ROLES OF VASOPRESSIN AND ENDOTHELIN IN DEOXYCORTICOSTERONE ACETATE-SALT HYPERTENSIVE RATS

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ABSTRACT

The contributions and interactions of the vasopressin (AVP) and endothelin (ET) systems in the hypertensive state were investigated in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The first part of the thesis reports on studies designed to assess the hemodynamic contribution of ET and to compare the responses of a non-selective ET antagonist to a selective ET_A antagonist. The second part of the thesis reports on experiments designed to evaluate the involvement of V_1 receptors and the reciprocal role of AVP and ET to the maintenance of blood pressure (BP). The third part of the thesis reports on studies designed to quantify the contribution of ET to the responses evoked by AVP. All the experiments in this thesis were carried out in chronically instrumented DOCA-salt hypertensive and SHAM control rats implanted with telemetry devices for recording of BP and Transonic flowprobes for recording of cardiac output (CO). Total peripheral resistance (TPR) was calculated from the BP and CO recordings.

In the first study, bosentan, a non-selective ET antagonist, and BMS-182874, a selective ET_A antagonist, were injected into both DOCA-salt hypertensive and SHAM rats intravenously. In contrast to SHAM rats, bosentan and BMS-182874 lowered BP and TPR dramatically in DOCA-salt hypertensive rats. Both antagonists increased CO. The magnitude and time-course of the hemodynamic effects of bosentan and BMS-182874 were similar. These results suggest that the contribution of ET to the maintenance of high BP in this hypertensive animal is exerted at the level of the resistance vessels. This contribution can be accounted for by its effects on ET_A receptors.

In the second study, [d(CH_2)$_5^1$, O-Me-Tyr^2,Arg^8]-vasopressin, a V_1 antagonist, was administered in the absence and presence of bosentan. Administration of the V_1 receptor antagonist alone failed to lower BP and TPR in DOCA-salt hypertensive rats. However, the V_1 antagonist did lower BP and TPR when the ET system had been blocked. In other experiments, bosentan was administered in the absence and presence of the V_1 antagonist. Bosentan alone reduced BP and TPR, but the responses to bosentan were exaggerated when V_1 receptors were blocked. These results indicate that AVP contributes to the maintenance of hypertension via its V_1 receptor-mediated vasoconstrictor effects. It also reveals the reciprocal or redundant nature of the AVP and ET systems in regulating hemodynamics in DOCA-salt hypertensive rats.

In the third study, a range of doses of AVP was infused intravenously in DOCA-salt hypertensive and SHAM rats before or after bosentan treatment. The increases in BP and TPR elicited by AVP were blunted by bosentan both in DOCA-salt hypertensive and SHAM rats. The effect of bosentan was more pronounced in the hypertensive rats. In contrast to AVP, responses to Ang II were not modified by bosentan. These findings provide direct evidence that ET contributes to the hemodynamic responses of AVP. This contribution to the pressor activity of AVP is exerted at the level of the resistance function of the circulation.

In conclusion, the results of this thesis demonstrate the involvement of both the ET and AVP systems and their reciprocal or redundant relationship in the control of systemic hemodynamics in the DOCA-salt model of hypertension. The results also demonstrate a contribution of ET to the pressor activity of AVP in this model.

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This thesis is dedicated with love to my parents:

Qinan Yu and Chaiwen Wu

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

ADH	antidiuretic hormone
AMP	adenosine monophosphate
Ang II	angiotensin II
ANP	atrial natriuretic peptide
ANS	autonomic nerve system
AP	area postrema
AQP	aquaporin
AVP	arginine vasopressin
BP	blood pressure
Ca^{2+}	calcium
cAMP	cyclic AMP
CO	cardiac output
dDAVP	1-desamino-8-D-arginine vasopressin
DG	diacylglycerol
DI rat	diabetes insipidus rat, a AVP-deficient Brattleboro rat
DOCA	deoxycorticosterone acetate
ECE	endothelin converting enzyme
EDCF	endothelial-derived constricting factor
ET	endothelin
ET_A	endothelin type A receptor

ET_B	endothelin type B receptor
HR	heart rate
icv	intracerebroventricular
IP3	inositol 1,4,5-triphosphate
iv	intravenous
K^+	potassium
L-NAME	N^w-nitro-L arginine-methyl ester
L-NMMA	NG-monomethyl-L-arginine
L-NNA	N^w-nitro-L-arginine
LSNA	lumbar sympathetic nerve activity
MX	methoxamine
Na^+	sodium
NE	norepinephrine
NO	nitric oxide
NOS	nitric oxide synthetase
NTS	nucleus tractus solitarius
PE	phenylephrine
PG	prostaglandin
PGI_2	prostacyclin
PKA	protein kinase A
PLC	phospholipases C
RSNA	renal sympathetic nerve activity

SAD	sinoaortic baroreceptors denervation
SD rat	Sprague Dawley rat
SHR	spontaneously hypertensive rat
SHRDI	offsprings of crossbreeding SHRSP with DI rats
SHR-SP	SHR stroke prone strain
TGR	Transgenic rat
TPC	total peripheral conductance
TPR	total peripheral resistance
V_1	vasopressin type I receptor
V_2	vasopressin type II receptor
WAP	AVP withdrawal induced antihypertensive phenomenon
WKY rat	Wistar-Kyoto rat

1. Introduction

Evidence has suggested that both the vasopressin (AVP) system and endothelin (ET) system play important roles in the development and maintenance of deoxycorticosterone acetate (DOCA)-salt hypertension. Both AVP and ET exert vasoconstrictor and hypertrophic effects on cardiovascular tissue. In this introduction, effects of AVP, ET and their interaction are reviewed in normotensive and hypertensive animals. In addition, the pathophysiology of DOCA-salt hypertension and the roles of AVP and ET in this hypertension are reviewed.

1.1 Cardiovascular Effects of AVP and ET in Normal Animals

1.1.1 Effect of AVP

Arginine vasopressin (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-GlyNH_2) is a polypeptide containing nine amino acids. It is secreted by the posterior pituitary gland, also called the neuro-hypophysis, which is composed mainly of glial-like cells called pituicytes (Guyton and Hall, 1996). However, the pituicytes do not secrete hormones; rather they act as a supporting structure for large numbers of terminal nerve fibers and terminal nerve endings arising from nerve tracts in the supraoptic and paraventricular nuclei of the hypothalamus. These tracts pass to the posterior pituitary gland through the pituitary stalk (hypophysial stalk). The nerve endings contain many secretory granules

which secrete the two posterior pituitary hormones, vasopressin and oxytocin. AVP is mainly synthesized in the cell bodies of the supraoptic nuclei, whereas oxytocin is synthesized in the paraventricular nuclei. Then they are transported in combination with carrier proteins called neurophysins down to the nerve endings in the posterior pituitary gland.

The release of AVP from the posterior pituitary occurs in response to an action potential. The generation of electrical signals and subsequent hormonal release occurs in response to synaptic release of neuroactive agents, such as acetylcholine and ET (see section 1.2.3.1), from nerve terminals in the hypothalamus and the posterior pituitary. When nerve impulses are transmitted downward along the fibers from the supraoptic or paraventricular nuclei, AVP is immediately released from the secretory granules in the nerve endings and absorbed into adjacent capillaries. Both the neurophysin and the hormone are secreted together, but because they are only loosely bound to each other, AVP separates almost immediately. Neurophysin has no known function after leaving the nerve terminals.

The historical development of AVP can be traced back to the last century. As reviewed by Cowley and Liard (Cowley and Liard, 1987), Oliver and Schafer first observed in 1895 that pituitary gland extracts caused a sustained increase in blood pressure (BP) when they were injected into anesthetized dogs (Oliver and Shafer, 1895). Three years later, Howell confirmed these observations and demonstrated that the pressor principle resided in extracts of the posterior lobe of the pituitary gland (Howell, 1898). The antidiuretic actions of AVP were discovered almost 20 years later. In 1913

two physicians, Farini and Von den Velden independently demonstrated the successful treatment of patients with diabetes insipidus with posterior pituitary extracts (Farini, 1913; Von den Velden, 1913). Subsequently, in 1924 Starling and Verney demonstrated the antidiuretic effects of posterior pituitary extracts on the isolated kidney (Starling and Verney, 1924). Because its pressor actions required amounts far in excess of those required for maximal antidiuretic activities (McConnaughey et al. 1999), the antidiuretic role of AVP became the predominant aspect of interest.

Nevertheless, the cardiovascular activity of AVP was revealed over a number of years. In 1922, Krogh showed that hypophysectomy was associated with vasodilation in the frog, suggesting a pituitary substance may play a role in cardiovascular regulation (Krogh, 1922). In addition, Frieden and Keller found dogs with diabetes insipidus were more susceptible to hemorrhage than normal dogs, suggesting the AVP might have an important role in the control of BP during pathological conditions, such as hemorrhage (Frieden and Keller, 1954). In 1956, Wagner and Braunwald demonstrated that much lower doses of AVP were required to exert vasoconstrictor responses in patients with autonomic nervous system insufficiency (Wagner and Braunwald, 1956). Unfortunately, many of these earlier observations were overlooked due to the overwhelming interest in the renal actions of AVP.

The cardiovascular and vascular smooth muscle actions of AVP were studied more intensively following several developments: the characterization and synthesis of AVP by Du Vigneaud in 1954, which indicated that the vasopressor and antidiuretic properties were in the same peptide (Du Vigneaud et al. 1954); the development of a

sensitive and specific radio immunoassay for measuring the concentration of AVP in plasma, urine and tissue (Miller and Moses, 1969; Mohring and Mohring, 1975); and the development of AVP analogues which selectively mimicked or antagonized the vasoconstrictive or antidiuretic activities of the peptide (Liard, 1988; Manning and Sawyer, 1986; Sawyer and Manning, 1988). The physiological range of plasma AVP is between 0.3 pg/ml, with water overhydration averaging 20 ml/kg body weight, to levels between 20 and 30 pg/ml with 48-hour water restriction (Cowley and Barber, 1983). These levels can increase to levels exceeding 600 pg/ml under certain stressful events, such as surgery and hypovolemia (Equivalence between units used in literature for AVP: 1 fmole=1.084 pg=0.434 μU; molecular weight of AVP: 1084).

AVP exerts its major biological actions through activation of V_1- and V_2 receptors. (Jard, 1998; Mayinger and Hensen, 1999). The V_1 receptor is mainly expressed in the liver and vascular smooth muscle cells where it mediates glycogenolytic and vasoconstrictor responses. The V_1 receptor is coupled to G protein $G_{q/11}$. Activation of this complex evokes increases in the activities of phospholipases C (PLC), D and A_2, production of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DG), activation of protein kinase C (PKC), mobilization of intracellular calcium and the influx of extracellular calcium via a receptor-operated Ca^{2+} channel. The V_2 receptor is expressed in the renal medulla, where it mediates the antidiuretic effect of AVP. The V_2 receptor is coupled to G protein Gs. Activation of this complex evokes increases in adenylyl cyclase activity, production of cyclic AMP (cAMP) and activation of protein kinase A (PKA). This signal transduction pathway mediates the insertion of water channels into

the luminal surface of the renal collecting tubule cells. More recently, another AVP receptor was demonstrated and named as V_3 or V_{1b} receptor (as opposed to the V_1 receptor, which has been reclassified as the V_{1a} receptor) (Nakamura et al. 1999; Jard et al. 1986). It is mainly expressed in corticotrophic cells in adenohypophysis and induces ACTH release.

1.1.1.1 Pressor properties of AVP

AVP may increase BP via its direct vasoconstrictor actions on vascular smooth muscle, antidiuretic actions on kidney and central actions on brain.

1.1.1.1.1 Vascular actions

AVP evokes vasoconstriction directly via V_1 receptors expressed on vascular smooth muscle cells. Direct observation of blood vessels has allowed investigators to evaluate the effective vasoconstrictor concentrations of AVP by measuring the effect of the peptide on lumen diameter. In 1973, Altura observed the vasoconstriction in rat mesenteric arterioles *in vivo* elicited by topically applying a very low concentration of AVP at 10 pg/ml, a concentration within the physiological range (Altura, 1973). These arterioles were more sensitive to AVP than to angiotensin II (Ang II). The threshold for Ang II was three times greater than that for AVP, which suggested that AVP might be one of the most potent circulating vasoconstrictors found at that time (Altura and Altura, 1977; Altura and Altura, 1984). Similarly, rat mesenteric arterioles were three to four orders of magnitude more sensitive to AVP than to epinephrine or norepinephrine (NE). Even rat aorta exhibited greater sensitivity to AVP than to Ang II (Altura and Altura,

1977; Altura, 1978). More recently, AVP has been reported to induce potent mitogenic and hypertrophic effects on vascular smooth muscle (Tahara et al. 1997; Serradeil-Le Gal et al. 1995). These effects were significantly inhibited by a V_1 receptor antagonist, suggesting the hyperplastic and hypertrophic actions of AVP are mainly mediated via its V_1 receptors on vascular smooth muscle cells.

The vasoconstrictor effects are not homogeneous. The most sensitive vessels to AVP are the mesenteric, skeletal muscle, skin and carotid arteries (Altura, 1975; Schmid and Patel, 1987), while hepatic arteries and veins are moderately sensitive. The sensitivity of vascular smooth muscle increases with decreasing vessel diameter. In 1995, Chen et al. reported that the sensitivity and maximal response to AVP was increased in small artery compared to conduit vessel: the rank order was tail artery > mesenteric artery > aorta (Chen et al. 1995a). However, the responses to AVP on coronary and cerebral arteries and on the pulmonary vascular bed are controversial (Altura and Altura, 1984). The peptide has been demonstrated to induce vasoconstriction and dilation of cerebral arteries (Lluch et al. 1984; Onoue et al. 1994; Armstead, 1996), no effect or dilation in pulmonary vasculature (Garcia-Villalon et al. 1996; Walker et al. 1989) and constriction and dilation of coronary arteries (Garcia-Villalon et al. 1996; Katusic et al. 1984; Okamura et al. 1999). The reasons for these differences are unknown, and they may be related to variation among animal species and among experimental procedures, as well as to the role of the endothelium in vascular reactivity. The AVP-induced vasodilatation is dependent on the integrity of the endothelium and is

associated with the release of nitric oxide (NO) (Katusic et al. 1984; Katusic, 1992; Okamura et al. 1999).

The pressor sensitivity to AVP is significantly different among species (Cowley and Liard, 1987). When the autonomic nerve system (ANS) is functional, humans seem to be the least sensitive, followed by dogs and then rats. With an intact ANS, a rise of only 10 mmHg BP was observed in humans at infusion rates of 100 ng/kg/min of AVP, whereas BP rose from 15-25 mmHg in dogs and rats at rates of only 10 ng/kg/min, indicating a 10-fold difference in pressor sensitivity.

In summary, AVP is a potent vasoconstrictor of resistance vessels from some of the major vascular beds. However, its effects are heterogeneous among various regional vascular beds and among species.

1.1.1.1.2 Effect on baroreflexes

AVP is a more potent vasoconstrictor of microvessels than Ang II. However, Ang II produces more potent pressor responses than AVP with an intact baroreceptor reflex arc (Schmid et al. 1985a). Studies have suggested that the pressor effects of AVP are strongly buffered by baroreflexes and the ANS. In 1974, Cowley et al. reported the substantial increases in AVP pressor sensitivity in conscious dogs with the sinoaortic baroreceptor denervation (SAD) (Cowley et al. 1974). The threshold sensitivity was reduced nearly 11-fold and the pressor sensitivity was 60-100 fold above normal conscious dogs. Infusion of physiological levels of AVP (0.2-2.0 mU/kg/min) caused an average increase in BP of 33 mmHg in SAD dogs compared with an increase of 5 mmHg in normal dogs. By comparison, only 3 fold increases in pressor sensitivity were

observed with infusions of NE. A similar enhancement of AVP pressor sensitivity was observed in conscious SAD rabbits (Undesser et al. 1985). These data indicate that AVP normally enhances the ability of the baroreflexes to buffer an AVP-induced rise of arterial pressure. Furthermore, in total areflexic dogs in which all CNS structures were removed, AVP induced an 8000-fold increase in pressor sensitivity when compared to normal conscious dogs (Cowley et al. 1974). Similarly, in humans with primary autonomic insufficiency, pressor responses to AVP were enhanced at least 50-fold compared to normal individuals, whereas the responses to both NE and Ang II were enhanced only 2-10 times (Mohring et al. 1980). Thus, the pressor responses to AVP are enhanced in both baroreceptor denervation or areflexic states in humans and dogs.

A different picture is seen in rats. AVP exerted only a 5-fold enhancement of pressor sensitivity, an enhancement which was similar to that for phenylephrine (PE) (5 fold) or Ang II (6 fold) in sinoaortic barodenervated rats (Webb et al. 1986). However, in 1987 Osborn et al. showed that the pressor sensitivity to AVP was markedly enhanced by ganglionic blockade, in contrast to Ang II in rats. The ANS was blocked by hexamethonium and methscopolamine and BP was supported by continuous infusion of NE. Under these conditions, AVP pressor sensitivity was increased 60 fold compared with a 2-8 fold enhancement to Ang II, which was similar to the responses of Ang II in SAD rats (Webb et al. 1986). The differences in AVP pressor sensitivity between SAD rats and rats with total autonomic blockade are the result of "nonbaroreflex" reduction of sympathetic tone by AVP. Rats in which the afferent limb of the baroreflex is blocked, but in which efferent sympathetic pathways are intact (such as SAD rats), have some

capacity to offset the vasoconstrictor actions of AVP by withdrawal of sympathetic tone. Pharmacological blockade of these efferent pathways (such as ganglionic blockade rats) removes this "nonbaroreflex" buffering mechanism and results in a further potentiation of the pressor activity of AVP.

Direct evidence for AVP modification of overall baroreflex function was tested by Cowley and his colleagues in 1984 using carotid sinus perfusion methods in anesthetized dogs (Cowley et al. 1984a). In these studies, the carotid sinus regions of both left and right internal carotid arteries were isolated from the rest of the systemic circulation, and carotid sinus pressure was controlled independently of systemic arterial pressure. Changes in aortic pressure and cardiac output were measured in response to step changes of blood pressure within the isolated carotid region. In contrast to elevated plasma levels of Ang II, they observed that elevated plasma AVP concentrations (from 51-455 pg/ml) enhanced reflex control of arterial pressure during decreases (60-105 mmHg) but not increases from the equilibrium point of carotid sinus pressure. The ratio of the change of aortic pressure to the change of carotid sinus pressure represents the strength of the reflex response and is defined as the "open-loop feedback gain" of the reflex, which was enhanced about 2 fold by the elevated plasma AVP concentrations. Elevation of aortic pressure expected from the vasoconstrictor actions of AVP were buffered by a second major mechanism, namely reductions in cardiac output (CO). Suppression of CO did not occur in conscious SAD dogs (Montani et al. 1980). These data indicate that AVP enhances the ability of the carotid reflexes to normalize decreases

of BP through changes in TPR, but buffers a rise in pressure from its own vasoconstrictor properties by initiating a fall of CO.

Changes in heart rate (HR) are often used as another index of baroreceptor activation. In conscious dogs, the bradycardic responses with AVP administration were greater than for equipressor doses of methoxamine, Ang II and PE (Hendrickx et al. 1976; Robinson, 1986). Enhanced bradycardic responses associated with AVP have also been demonstrated in rabbits (Undesser et al. 1985) and rats (Datar et al. 1985; Patel, 1991). Therefore, the reflex-induced bradycardia observed with administration of AVP appears at least to be consistent between species and is far greater than that observed with other vasoconstrictor agents. Thus, as judged from change in HR, these data clearly suggest that AVP can augment baroreflexes in a specific way.

Recording of peripheral sympathetic nerve activity provides direct evidence on the interaction of AVP with baroreflex control. Undesser et al. recorded renal sympathetic nerve activity (RSNA) in conscious rabbits during equipressor doses of AVP and PE (Undesser et al. 1985). They found that the decrease in RSNA was much greater with AVP. The effect of the decrease in RSNA by AVP was significantly attenuated by vagotomy and SAD, indicating that the reduction of RSNA was dependent on afferent baroreceptor activity. Similarly, in anesthetized rabbits, AVP was found to facilitate reflex inhibition of lumbar sympathetic nerve activity (LSNA) (Sharabi et al. 1985). These results contrast with those obtained with Ang II, which produced less change in LSNA than PE for similar pressor responses (Guo and Abboud, 1984). Thus, at least in rabbits, baroreflex inhibition of peripheral sympathetic nerve activity is

enhanced during AVP administration and inhibited with Ang II administration. However, in anesthetized rats, AVP did not facilitate baroreflex inhibition of LSNA (Sharabi et al. 1985), but it did have such an effect on HR (Schmid et al. 1985b). Thus, AVP appears to facilitate reflex inhibition of HR and peripheral sympathetic activity, but species differences are evident.

AVP may facilitate baroreflex activity at several sites. In 1985, Schmid et al. used the isolated carotid sinus preparation of the rabbit to investigate the effects of AVP on carotid baroreceptor-induced inhibition of LSNA (Schmid et al. 1985a). They found that this peptide enhanced reflexes both when it was restricted to the region of the carotid sinus and when it was excluded from this region. They suggested that the latter effect was due to an action on brain. In 1981, Liard et al. reported that infusions of AVP into the canine vertebral artery appeared to elicit greater reflex decreases in HR than similar intravenous infusions, suggesting that AVP may act on sites within the area of perfusion of the vertebral artery, such as the area postrema (AP) (Liard et al. 1981).

The AP is located on the dorsal surface of the fourth ventricle of the medulla, and it lacks a blood-brain barrier (Wislocki and Leduc, 1952). Therefore, it would be readily accessible to blood borne AVP. The AP is rich in AVP receptors (Phillips et al. 1988b; Phillips et al. 1988a). The enhancement of sensitivity of baroreflexes by AVP was absent in AP-lesioned animals (Undesser et al. 1985; Peuler et al. 1990). In AP-lesioned rats and rabbits, the differences in reflex bradycardic responses to AVP vs PE disappeared. In animals in which the cell bodies of the AP had been destroyed by injection of an excitotoxin or by AVP antagonists, the AVP-induced enhancement of baroreflexes was

blocked (Cox et al. 1990; Stebbins et al. 1998). Many neurons originating in the AP project into the nucleus tractus solitarius (NTS), where nerve endings of baroreceptor afferents also terminate. Therefore, activation of AP neurons by AVP, either from microinjections into the AP or by intravenous infusions, alters the response of some NTS neurons to baroreflex stimulation (Qu et al. 1997).

The AVP-induced enhancement of baroreflex function appears to be mediated by V_1 receptors in the AP (Hasser and Bishop, 1990; Stebbins et al. 1998). In 1990, Hasser and Bishop reported that the ability of circulating AVP to augment baroreflex inhibition of RSNA compared to PE was abolished by microinjection of a V_1 antagonist into the AP in anesthetized rabbits (Hasser and Bishop, 1990). Similarly, Stebbins et al. observed the microinjection of a V_1 antagonist into the AP attenuated AVP enhanced baroreflex inhibition in anesthetized cats (Stebbins et al. 1998). However, the role of V_1 receptors in enhancement of baroreflexes by AVP in rats is less clear. In anesthetized rats, microinjection of AVP into the AP caused an increase in BP. The increased BP by AVP was attenuated by the microinjection of a V_1 antagonist into this area (Lowes et al. 1993; Smith et al. 1994). In addition, V_2 receptors may also be involved in the AVP-induced enhancement of baroreflexes in rats. In the Brattleboro rat, a rat homozygous for diabetes insipidus (DI), the impaired baroreflex function was restored to normal by intravenous injection of DDAVP, a V_2 receptor agonist (Imai et al. 1983). Similarly, intravenous infusion of a V_2 antagonist shifted the baroreflex curve (pulse interval vs. BP) to a lower sensitivity, while a V_1 antagonist shifted the curve to higher sensitivity in conscious rats (Unger et al. 1986b). Additionally, microinjection of DDAVP directly into

the AP had a depressor effect in anesthetized rats (Lowes et al. 1993). Taken together, it has been concluded that AVP can sensitize the baroreflex by acting on both V_1 and V_2 receptors accessible from the blood and there may be species differences in term of receptor involvement.

In summary, AVP appears to enhance baroreflex sensitivity to a greater extent than other pressor agents. It interacts with baroreceptors (afferent), the AP (CNS) or efferent limbs of the reflex arc to facilitate baroreflex inhibition of autonomic nervous system (ANS) function to buffer its cardiovascular effects. Species differences may exist with respect to baroreflex modulation by AVP and to the receptor systems involved.

1.1.1.1.3 Effects on cardiac output and total peripheral resistance

As elucidated to above, the unimpressive pressor effects of AVP are buffered by another major mechanism, i.e. a decrease in CO (Cowley et al. 1984a). Indeed, plasma concentrations required to increase BP exceed 50 to 100 pg/ml in man and dogs, concentrations which are well above the physiological range (Aylward et al. 1986; Montani et al. 1980). Rats do appear to be more sensitive to the pressor effects of AVP, and marked elevations of BP have been reported to occur at plasma concentration of 20 pg/kg (Datar et al. 1985; Osborn et al. 1987).

In contrast to changes in BP, marked decreases of CO occur at plasma levels of AVP well within the physiological range. In 1980, Montani et al. reported that elevations in the circulating levels of AVP in conscious dogs to only 4.2 pg/ml from a control value of 2.1 pg/ml were associated with significant 8% decreases in CO and 12% increases in TPR, even though there were no changes in BP (Montani et al. 1980).

These results demonstrated clearly that plasma concentrations of AVP within the physiological range can significantly decrease CO and increase systemic vascular resistance. With increments in the concentrations of the peptide to about 71 pg/ml, a 57% increase in TPR was accompanied by a 24% decrease in CO resulting in only an 8 mmHg increase in BP. Similarly, in humans, an increased plasma AVP level by 13 pg/ml from a control of 5 pg/ml resulted in a 15% decrease in CO and a 20% increase in TPR without marked changes in BP (Ebert et al. 1986). Because BP is the product of CO and TPR, the vasoconstrictor effects of the peptide can conceivably be buffered directly at the level of vascular smooth muscle via changes in TPR or indirectly via changes in CO. Rats appear to be the least sensitive to these effects, since elevation of the plasma levels of AVP to approximately 30 pg/ml evoked a 23% rise in TPR, but only a 9% fall in CO, resulting in a 13 mmHg increase in BP in conscious rats (Osborn et al. 1987). But the highest dose of AVP increased BP 34%, whereas TPR was increased 150% and CO was decreased 42% in rats. Similar results have been demonstrated in rats and dogs where endogenous AVP secretion has been stimulated by dehydration or hypertonic saline administration (Charocopos et al. 1982; Montani et al. 1980; Tipayamontri et al. 1987). In the pathophysiological range, AVP induced increases in both BP and TPR, but the elevations in BP are not proportional to the rises in TPR. Five and 17% increases in BP were associated with 54 and 94% increases in TPR in dogs (Montani et al. 1980; Pang et al. 1979).

Direct comparisons of the hemodynamic effects of AVP and Ang II have been studied in conscious dogs. Heyndrickx et al. reported that for 25% elevations in BP, CO

fell by 44% with AVP, 16% with methoxamine (MX) and 8% with Ang II, while TPR rose by 153% with AVP, 61% with MX and 48% with Ang II in conscious dogs (Hendrickx et al. 1976). Even in rats, CO fell and TPR rose greater with AVP than Ang II for comparable increases of BP (Osborn et al. 1987). Therefore, AVP appears to exert greater effects on CO and TPR for a given increase in BP than other vasoconstrictors.

Baroreflex control of capacitance function may contribute to the fall of CO induced by AVP. The decreases in CO associated with the elevation of AVP in the animal with intact reflexes were markedly reduced in the denervated dogs (Montani et al. 1980). Moreover, ganglionic blockade also attenuated the CO responses to AVP (FuJii and Vatner, 1987). The AVP -induced fall of CO was nearly absent in barodenervated dogs, despite substantial elevations of TPR. Cowley and Barber analyzed these results and indicated that the unexpectedly small influence of AVP on CO in the barodenervated state during graded increases in TPR could be explained by the hydraulic characteristic of the parallel nature of the vessels of the systemic circulation (Cowley and Barber, 1983). Parallel flow beds can exhibit increases of TPR with no decrease in CO if one of the parallel beds has a greater vascular compliance (skin or splanchnic) than another. However, in conscious rats, the impairment of reflex function by either ganglion blockade or SAD did not attenuate the decreases in CO but potentiated the increases in both BP and TPR associated with AVP administration (Osborn et al. 1987; Webb et al. 1986).

AVP could have negative inotropic actions on heart. In conscious dogs, AVP depressed left ventricular performance at plasma concentrations of 15 pg/ml. This

negative inotropic effect was present after autonomic blockade and was reversed by a V_1 antagonist (Cheng et al. 1993). However, in rats, AVP exerted coronary vasoconstriction and positive inotropic effects on heart at concentrations between 50 and 100 pg/ml and myocardial depression at higher concentrations (Walker et al. 1988; Boyle and Segal, 1986). By using the isolated heart perfusion technique, Graf et al. found that AVP at high concentrations of 120 - 2500 pg/ml caused myocardial depression indirectly by reducing coronary flow during constant perfusion pressure in guinea pig, and it had no direct myocardial effect because when perfusion was maintained by constant coronary flow, myocardial function was maintained (Graf et al. 1997). In view of these considerations, it is unlikely that a direct negative inotropic action of AVP contributes substantially to the fall of CO.

The cardiovascular effects of AVP could be buffered by decreases in CO that were mediated via changes in capacitance function of a highly compliant vascular bed. Changes in capacitance have marked effects on venous return and thus, play an important role in regulating CO (Greenway and Lautt, 1986). Indeed, AVP infusions were accompanied by a decrease in venous return in dogs (Tipayamontri et al. 1987; Emerson, 1966) and pigs (Welt and Rutlen, 1991). In 1990, Martin and McNeill directly assessed the effects of AVP on capacitance function in anesthetized cats by draining blood from superior and inferior venae cava into an external reservoir and then returning blood to the right atrium at a constant rate (Martin and McNeill, 1990). In contrast to Ang II and NE, AVP was associated with concentration-dependent increases in whole body capacitance, and this increase was accounted for by a reflex increase in unstressed

vascular volume but not systemic compliance, supporting the theory advanced by Cowley and Barber (1983). However, AVP failed to change mean circulatory filling pressure and the pressure gradient for venous return in rats (Pang and Tabrizchi, 1986; Hernandez et al. 1994). Regardless of the precise mechanism, it is clear that CO is dramatically decreased during AVP infusion and that these changes in CO may attenuate the pressor effects of the peptide.

In summary, AVP evokes a marked fall in CO compared to equipressor doses of other vasoconstrictors, especially in dogs and humans. The fall in CO appears to be mediated by enhanced cardiovascular reflexes, largely at the level of the capacitance vessels although a negative inotropic effect on heart cannot be excluded. In rats, the fall in CO is less and the mechanism of the fall in CO has not been determined.

1.1.1.1.4 Effects on kidney

Functional studies have shown that the water retaining properties of AVP are mainly mediated via V_2 receptors located in the collecting tubule and the thick ascending limb of Henle's loop. The major effect of AVP on the kidney is to increase water, but not urea, permeability of the late distal tubules and collecting ducts in renal cortex and outer medulla (Valtin, 1987). However, AVP does increase urea as well as water permeability of the collecting duct that lies within the inner medulla (Jamison and Kriz, 1982; Sands et al. 1987). Consequently, urea can now diffuse out of the inner medullary collecting duct into the interstitium more effectively because its concentration within the tubular fluid was raised by the abstraction of water from the proximal portions of the collecting duct. Aside from these effects, AVP also enhances the reabsorption of NaCl

from the thick ascending limbs of Henle that runs through the outer medulla (Hebert et al. 1981). As the result of NaCl and urea reabsorption in outer and inner medulla, the peptide enhances the buildup of the corticopapillary gradient, which is critical for the antidiuretic effect of AVP at the collecting duct.

The discovery of the aquaporin (AQP) family of water channels has greatly improved the understanding of how water crosses epithelial cells (Marples et al. 1999). AQPs are a family of transmembrane channel proteins that serve to regulate transepithelial water reabsorption and body fluid homeostasis. AQP1, the first AQP to be characterized (Agre et al. 1993), is involved in water movement of the proximal tubule, whereas AQP2, AQP3 and AQP4 are found in the collecting duct. AQP2 is the AVP-regulated water channel that is expressed exclusively in the principal cells of collecting duct (Agre et al. 1995). AVP binds to V_2 receptors in the basolateral membrane of collecting duct principal cells and induces the insertion of AQP2 from a store within intracellular vesicles into the apical plasma membrane of the cells, with cAMP as the second messenger (Marples et al. 1999). This action of AVP results in the enhancement of water reabsorption in the collecting duct.

In dogs, prolonged administration of AVP for 2-3 weeks increased BP with fixed water intake (Smith et al. 1979), but BP remained normal when water intake was servocontrolled (Cowley et al. 1984b). These findings indicate that the plasma levels of AVP in the range of 15-25 pg/ml, as measured in these studies, produces hypertension more related to expansion of body fluid volume than to any vasoconstrictor effect. This

is supported by the observation that continuous infusion of dDAVP, a V_2 receptor agonist, and hypotonic fluid induced an increase in BP in rats (Gross et al. 1982).

Recently, six lines of evidence supporting the causative role of the kidney in hypertension have been reviewed by Cowley and Roman (Cowley and Roman, 1996). The importance of small decreases of blood flow to the renal medulla was emphasized in the development and maintenance of hypertension. Chronic infusion of AVP failed to induce sustained hypertension. In contrast to AVP, chronic intravenous infusion of a selective V_1 receptor agonist induced sustained hypertension in the absence of water retention in conscious rats (Cowley et al. 1994). This effect could be prevented if a selective V_1 receptor antagonist was administered simultaneously into the renal medulla (Szczepanska-Sadowska et al. 1994). Therefore, the renal medulla appears to be a critical site for V_1 receptor-mediated hypertensive effects. Indeed, chronic infusion of a V_1 receptor agonist directly into renal medullary interstitial space resulted in a sustained decrease in renal medullary blood flow and increase in BP in conscious instrumented rats (Cowley et al. 1998). Together with the fact that the renal medullary vasculature is quite sensitive to small elevations of plasma AVP (6-12 pg/ml) (Franchini and Cowley, 1996), it is reasonable to postulate that V_1 receptor induced hypertension is at least in part related to its vasoconstrictor effect on renal medullary vasculature.

In summary, the major effects of AVP on the kidney are to increase the insertion of AQP2 into the apical membrane of the principal cell of collecting ducts via its V_2 receptor, and then to enhance water permeability in collecting ducts. The vasoconstrictor effect of V_1 receptors on renal medullary vasculature, which induces the reduction of

medullary flow and then the decreases in renal interstitial hydrostatic pressure and Na excretion, contributes at least in part to its hypertensive action.

1.1.1.1.5 Effects on brain

In addition to its central actions on memory formation, fever and social recognition (Alescio-Lautier et al. 1993), AVP exerts its cardiovascular effects centrally as well. Central infusions of AVP into certain areas, such as the nucleus of the solitary tract (NST) (Matsuguchi et al. 1982), the locus coeruleus (Berecek et al. 1984b) and the ventrolateral medulla (Andreatta-Van-Leyen et al. 1990), produce increases in BP and HR. Similar pressor responses were also observed in AVP-deficient Brattleboro rats, indicating that the pressor responses could not be accounted for by activation of endogenous brain AVP or neurohypophysial release of the hormone (Nussey et al. 1984). The pressor effect of centrally administered AVP was blocked by central but not peripheral administration of a V_1 receptor antagonist (Berecek et al. 1984a; Unger et al. 1986b).

In contrast to its peripheral actions, the central pressor effects of AVP appear to be associated with an enhancement of sympathetic activity, such as increases in HR, efferent splanchnic nerve activity and renal nerve activity (Rohmeiss et al. 1986). These responses were attenuated by intravenous injection of the α-adrenoceptor antagonist, phentolamine (Rohmeiss et al. 1986; Riphagen and Pittman, 1989), demonstrating that the central pressor effects of AVP are mediated at least in part by the activation of sympathetic activity. Moreover, AVP suppresses baroreflexes in normotensive animals centrally. Administration of a V_1 receptor antagonist centrally increased the sensitivity of

baroreflexes (Unger et al. 1986a). By contrast, intravenous infusion of the V_1 receptor antagonist had no effect on baroreflexes. These results suggest that AVP inhibits baroreflexes in the brain via its V_1 receptors, which are not accessible from the peripheral circulation.

In summary, the effects of AVP in certain areas of the brain are associated with increases in BP and HR. These actions are mediated by an increase in sympathetic nerve activity and a decrease in baroreflex sensitivity.

1.1.1.2 Depressor properties of AVP

In contrast to the pressor effects of AVP, there is mounting evidence that AVP may exert depressor responses in animals and humans through direct vasodilatation and effects on kidney and brain. The recent discovery of novel selective hypotensive vasopressin peptides has provided additional evidence supporting a depressor function (Chan et al. 1998).

1.1.1.2.1 Effects on vasculature

AVP induced vasodilatation has been reported both in animals and humans, and it is not clear which receptor subtype, V_1, V_2 or a distinctly different receptor subtype, mediates the vasodilating effects of AVP. In fact, both V_1 and V_2 receptors antagonists have been shown to inhibit the vasodilating action. In humans, Hirsch et al. and Imaizumi et al. reported that AVP in pharmacological doses caused vasodilation of vessels in the forearm (Hirsch et al. 1989; Imaizumi et al. 1992), and this vasodilation could be inhibited by a V_2 receptor antagonist. Further experiments showed that this

vasodilator effect was abolished by N^{w}-nitro-L arginine-methyl ester (L-NAME), a nitric oxide synthetase (NOS) inhibitor, indicating the effect depends on the release of NO (Tagawa et al. 1993). In dog, Liard observed a vasodilator effect of the systemic vasculature induced by dDAVP, a V_2 receptor agonist, and this effect was antagonized by L-NAME (Liard, 1994). A similar effect was also found in the rat aorta (Yamada et al. 1993). Intrarenal infusion of AVP (Naitoh et al. 1993) or AVP with a V_1 receptor antagonist (Aki et al. 1994) induced renal vasodilatation in dogs, and these effects were reduced by a V_2 receptor antagonist. Furthermore, this renal vasodilatation was also suppressed by a NOS inhibitor (Haynes et al. 1993). On the other hand, NO-releasing activity of AVP is also reportedly mediated by V_1 receptor stimulation. Katusic et al. showed that V_1 receptor stimulation relaxed the dog brain stem artery (Katusic, 1992). The relaxation was inhibited by NG-monomethyl-L-arginine (L-NMMA). In addition, AVP increased the production of cGMP, a second messenger of NO, in cultured porcine aortic endothelial cells through V_1 receptor stimulation (Schini et al. 1990). Furthermore, Walker et al. reported a V_1 receptor-mediated vasodilatation in the isolated rat lung during hypoxia (Walker et al. 1989). The controversy of AVP receptor subtype-inducing vasodilatation may be due to species differences and differences among arteries and regional beds. Briefly, AVP can induce vasodilatation and this effect may be NO-release dependent.

Recently, Chan et al. discovered four novel selective hypotensive AVP peptides (Chan et al. 1998), which could elicit a marked fall in BP in anesthetized rats. This effect was independent of the peripheral autonomic, bradykinin and prostaglandin system, and

was not mediated by the known classical AVP/OT receptors. NO did not appear to have an important role in their vasodepressor action. These results indicate that there may exist an unknown AVP receptor subtype or a new receptor outside the AVP receptor family for these novel hypotensive AVP peptides. The findings also suggest another mechanism for AVP-induced vasodilatation aside from the release of NO.

In summary, besides its vasoconstrictor properties, AVP can induce vasodilatation under certain conditions through V_1 or V_2 receptor. This vasodilator effect appears to be related to the release of NO. Additionally, a novel receptor subtype may evoke a NO independent vasodilation.

1.1.1.2.2 Effects on kidney

Despite the fact that AVP is an antidiuretic and antinatriuretic hormone *in vitro*, it does induce natriuresis when administered to animals for a long period *in vivo*. Studies done by Hall et al. showed that prolonged infusion of AVP normally produced water retention and increased urinary osmolarity during the first four days. After that, however, urine volume and osmolarity returned to normal despite the continued infusion of AVP (Hall et al. 1986). This phenomenon is often referred to as "escape" from the antidiuretic action of AVP. Moreover, AVP administration in conscious unrestrained rats increased sodium excretion (Wang and McNeill, 1994), despite reductions in urine flow (Brimble et al. 1988; Lote et al. 1989). After immunoneutralization of AVP, a posterior pituitary extract did not induce natriuresis in anesthetized saline loaded rats, as compared to untreated extract (Ponec et al. 1991), indicating that AVP may be the natriuretic substance in the posterior pituitary extract. The mechanism of the natriuresis induced by

AVP is not clear. Several possible explanations have been proposed, including pressure natriuresis (Hall et al. 1986), decreases in renin secretion (DiBona, 1982; Smith et al. 1979), increases in circulating atrial natriuretic peptide (ANP) (Manning et al. 1985) and increases in PG secretion (Schuster et al. 1984). In any case, regardless of the mechanism, AVP does have an effect to induce natriuresis in animals.

The relationship between the blood flow in the renal medulla, which lacks autoregulation (Roman et al. 1988; Mattson et al. 1993), and hypertension has been studied intensively by Cowley and his co-workers recently. In contrast to V_1 receptor agonists (see section 1.1.1.1.4), chronic intravenous infusion of AVP failed to induced sustained hypertension in conscious rats (Cowley et al. 1994). Furthermore, chronic infusion of a V_1 receptor agonist, but not AVP, directly into the renal medullary interstitial space induced a sustained decrease in renal medullary blood flow and an increase in BP in conscious instrumented rats (Cowley et al. 1998). These results indicate that some component of AVP may counteract its V_1 receptor effects on the renal medullary vasculature to limit the increase in BP and this component could be due to the stimulation of V_2 receptors by AVP in renal medulla or some unidentified vasodilator.

In summary, despite its antidiuretic effects, prolonged infusion of AVP induces natriuresis in animals. In contrast to a V_1 receptor agonist, AVP fails to induce hypertension when administered directly into the renal medulla, indicating that some component of AVP may counteract its V_1 receptor effect on renal medulla to limit BP elevation.

1.1.1.2.3 Effects on brain

It has been well established that central infusion of AVP in certain areas of the brain is associated with an elevation of BP and HR (See section 1.1.1.1.5). However, the peptide has been reported to lower BP and HR under certain conditions. Hemorrhage, which is known to release AVP into the cerebrospinal fluid (CSF) and brain tissue (Ota et al. 1994; Szczepanska-Sadowska et al. 1983), induces hypotension and bradycardia in normotensive rats. However, this response could be significantly attenuated by infusion of a V_1 receptor antagonist into the lateral cerebral ventricles (Budzikowski et al. 1996), indicating that a central V_1 receptor system is involved in hypovolemic hypotension and bradycardia. Indeed, mild, rapid hemorrhage (1 ml/100 g for 30 seconds) induced severe bradycardia and hypotension observed in Long-Evans rats, was absent in AVP deficient Brattleboro rats (Imai et al. 1996). Microinjection of AVP into the NTS at low doses (1-100 pg) caused a dose-dependent decrease in BP and HR in rats, and only high doses (1-10 ng) caused an increase in BP and HR (Sonntag et al. 1990; Brattstrom et al. 1988). Moreover, microinjection of AVP into the subfornical organ (SFO) resulted in significant decreases in BP; while microinjection of the peptide into the third ventricle produced large increases in BP (Smith and Ferguson, 1997). These results indicate that central hypotensive effects of AVP may be related to dose and location.

In 1983, Ganten et al. developed a new hypertensive model, which was established by cross-breeding SHR-SP with Brattleboro rats (Ganten et al. 1983). Their offspring (SHRDI) were hypertensive with undetectable plasma, hypothalamic and pituitary gland AVP levels. They concluded that AVP is not essential for the development and maintenance of spontaneous hypertension. However, another

interpretation is that it is the central deficiency of AVP that is necessary for the hypertensive state. Indeed, Brattleboro rats are hypertensive compared to control rats (Imai et al. 1990).

In summary, in addition to its central pressor effects, AVP exerts depressor actions in brain as well. The depressor actions may be related to dose and location, and the deficiency in this system may contribute to the hypertensive state. In addition, central AVP also contributes to the hypotension and bradycardia induced by hypovolemia.

1.1.1.2.4 The withdrawal-induced antihypertensive phenomenon

Cessation of a 3-hour infusion of AVP (20 ng/kg/min) induces a large fall in BP below pre-infusion basal levels in both SHR and DOCA-salt hypertensive rats, but not in control normotensive rats (Chiu and McNeill, 1985; Chiu and McNeill, 1989b; Chiu and McNeill, 1989a; Wang and McNeill, 1994; Balakrishnan and McNeill, 1996; Talom and McNeill, 1997). The response was named the "withdrawal-induced antihypertensive phenomenon (WAP)". The WAP is relatively specific to AVP (Chiu and McNeill, 1986), because equipressor doses of PE or Ang II caused no or much lower falls in BP. The fall in BP was dramatic (13-17 mmHg recorded by radiotelemetry devices and 30-50 mmHg recorded by externalized catheters) and lasted for 5 days (Balakrishnan and McNeill, 1996). The magnitude of the WAP paralleled the degree of hypertension (Chiu and McNeill, 1989b). Both V_1 receptor stimulation and the associated increases in BP were required for the phenomenon (Chiu and McNeill, 1987), since the WAP could be abolished by a V_1 receptor antagonist even when BP was elevated to a similar extent with PE or when BP elevation was prevented by pre-treatment with sodium

nitroprusside. These findings suggest that AVP possesses a blood pressure lowering property which is masked by its direct vasoconstrictor properties, and this BP lowering property in the WAP is mediated by its V_1 receptors.

The mechanism of the WAP is not clear yet. It may involve an interaction of AVP with autonomic function or an effect of AVP on renal function. Chiu and McNeill reported the WAP was exaggerated in SHR rats sympathectomized by chronic treatment with guanethidine (Chiu and McNeill, 1995). Moreover, a small drop in BP was also observed in sympathectomized WKY after the withdrawal of AVP. Together with the fact that AVP can sensitize baroreflexes and depress sympathetic activity (see section 1.1.1.1.2), these findings indicate that withdrawal of sympathetic activity is a contributing factor or a pre-requisite condition for development of the WAP. In other experiments, Wang et al. showed a large natriuresis during the AVP infusion in hypertensive rats. Furthermore, replacement of the sodium losses that occurred during the AVP infusion attenuated the WAP markedly in DOCA-salt hypertensive rats, but not in SHR (Wang and McNeill, 1994), suggesting that sodium losses contribute to the WAP in the DOCA-salt model but not in the SHR. Recently, Tatchum-Talom and McNeill tested the role of NO in the WAP, and found that WAP could be produced in N^{w}-nitro-L-arginine (L-NNA), a NOS inhibitor, induced hypertension (Talom and McNeill, 1997). L-NNA failed to prevent the WAP, and chronic treatment with L-arginine, a NO precursor, did not exaggerate the WAP in SHR. These findings suggest that the NO pathway is not essential to the WAP at least in SHR rats. Functional studies have showed

that the WAP was due mainly to decreases in CO and not to changes in TPR (Balakrishnan and McNeill, 1996).

In summary, cessation of a prolonged infusion of AVP is associated with a dramatic and prolonged fall in BP in hypertensive animals (SHR, DOCA-salt and L-NNA induced hypertensive rats) but not in normotensive rats. AVP appears to have a hypotensive property which counteracts its well-known blood pressure elevating properties.

1.1.2. Effects of ET

In 1988, endothelin-1 (ET-1) was isolated and identified from the supernatant of cultured porcine aortic endothelial cells as a potent vasoconstrictor and pressor substance (Yanagisawa et al. 1988). This peptide has a molecular weight of 2492. It consists of a 21-amino acid with two intrachain disulfide bridges which occur at fixed positions between residues 1 and 15, and 3 and 11, a hydrophobic tail and a terminal tryptophan residue. ET-1 is present in many mammalian species, including humans. Two additional human ET isopeptides, ET-2 and ET-3, are encoded by separate genes (Inoue et al. 1989). These isoforms of ET share the identical chemical features. The three ETs also have structural and functional similarities to the sarafotoxins, a family of isopeptides isolated from the venom of the snake *Atractaspis engaddensis*, which suggests a possible common evolutionary origin (Kloog and Sokolovsky, 1989).

The discovery of ET came from the identification of an endothelial-derived constricting factor (EDCF). In 1984, two abstracts were published, one by Agricola et al. and another by O'Brien and McMurtry (Agricola et al. 1984; O'Brien and McMurtry,

1984). These abstracts reported the discovery of a vasoconstrictor substance secreted from endothelial cells. In the following year, a paper by Hickey et al. (Hickey et al. 1985) appeared in which they concluded that this substance was a endothelial cell-derived polypeptide vasoconstrictor, since it was protease-sensitive, and caused a dose-dependent constrictor response in coronary arteries if extracellular Ca^{2+} was available. This vasoconstrictor was insensitive to α and β adrenergic, serotonergic, histaminergic and cholinergic receptor antagonists, as well as cyclooxygenase and lipoxygenase inhibitors. In 1986, Gillespie et al. studied the vasoconstrictor properties and first referred to it as "endothelial-derived constrictor factor" or "EDCF" (Gillespie et al. 1986). In the following year, another relevant paper was published from the same group (O'Brien et al. 1987), in which they confirmed the polypeptide nature of EDCF and estimated its molecular weight to be around 3000 daltons. Moreover, they noted that its constrictor effect was partially reversed by the calcium antagonist, verapamil. Finally in 1988, Yanagisawa et al. succeeded in sequencing this polypeptide from porcine aortic endothelial cells and named it as "endothelin" (Yanagisawa et al. 1988). They observed its remarkable potency as a vasoconstrictor agent, more potent than that of Ang II and AVP, making it the most potent mammalian vasoconstrictor polypeptide to be identified so far. They also confirmed Hickey's earlier observation relating to the Ca^{2+}-dependency of the coronary constrictor response and wrote their paper by concluding that an influx of Ca^{2+} is required for the action of ET.

The discovery of ET receptors and development of receptor antagonists accelerated investigation of the physiological and pathophysiological roles of the ETs.

The development of ET-deficient and ET receptor-deficient mice demonstrated the crucial roles in normal embryonic development. ET-1-deficient mice have craniofacial and cardiac abnormalities and die of respiratory failure soon after birth (Kurihara et al. 1994). A deficiency in ET_B receptors results in aganglionic megacolon, which resembles Hirschsprung's disease in human (Puffenberger et al. 1994).

1.1.2.1 ET biosynthesis and its receptors

Although vascular endothelial cells are the major source of ET-1, the genes that encode the three endothelin isopeptides are expressed in a wide variety of cell types, including cardiac myocytes, vascular smooth muscle, renal tubular epithelium, glomerular mesangium, glia, the pituitary, macrophages, etc., which suggests that the peptides may participate in complex regulatory mechanisms in various organs (Inoue et al. 1989; Sakurai et al. 1991).

ET isoforms are synthesized from large pre-proforms (preproET-1: human 212 amino acids, porcine 203 amino acids) which undergo post-translational processing by dibasic amino acid endopeptidases to pro-peptides or big ETs (Big ET-1: human 38 amino acids, porcine 39 amino acids) (Yanagisawa et al. 1988; Kido et al. 1997). Big ET is then cleaved by ET converting enzyme (ECE), a phosphoramidon-sensitive membrane bound metalloproteinase (Ohnaka et al. 1993), to yield the final 21-amino acid peptide. In vascular endothelial cells, ET-1 is secreted via the constitutive pathway, and the rate-limiting step of its biosynthesis is thought to be at the level of transcription (Yanagisawa et al. 1988). The conversion of big ET-1 to ET-1 is essential for biological activity, because the pressor action of big ET-1 was almost completely inhibited by a relatively

large dose of phosphoramidon, an inhibitor of ECE (Gardiner et al. 1991). Indeed, ET-1 is 140-fold more potent as a vasoconstrictor compared to the precursor peptide (Kimura et al. 1989).

ET-1 gene expression in vascular endothelial cells is regulated by a variety of physical and chemical stimuli. ET-1 mRNA level is increased after treatment of endothelial cells with growth factors (Kurihara et al. 1989), insulin, or with vasoactive substances such as NE, Ang II, AVP and bradykinin (Imai et al. 1992). High shear stress (25 dynes/cm^2) decreases ET mRNA levels, whereas low shear stress (5 dynes/cm^2) increases its mRNA expression (Malek and Izumo, 1992; Sumpio and Widmann, 1990). In contrast, the expression of ET-1 mRNA is inhibited by stimuli that act to increase intracellular levels of cGMP, including endothelium-derived relaxing factor (NO), prostacyclin, ANP, heparin and adrenomedullin (Emori et al. 1993; Imai et al. 1993; Kohno et al. 1995).

ET-induced responses may be divided into two groups: the first group including vasoconstriction, bronchoconstriction, uterine smooth muscle contraction and stimulation of aldosterone secretion; and the second group including endothelium-dependent vasorelaxation and inhibition of platelet aggregation. These observations suggest that at least two distinct ET receptors mediate these pharmacological responses. Indeed, two similar but distinct cDNA encoding ET receptors were cloned from rats, bovine, and humans (Arai et al. 1990; Lin et al. 1991; Sakurai et al. 1990; Sakamoto et al. 1991). These receptors can be classified into two groups, designated as ET_A and ET_B, according to the relative binding affinities of the three ET isopeptides for the receptors.

The order of affinity for the ET_A receptor is ET-1 > ET-2 > ET-3 while the ET_B receptor binds to the three peptides with equal affinity (Sakurai et al. 1992). ET_A receptors are present on vascular smooth muscle cells and mediate vasoconstriction and proliferation in response to ET-1 (Ohlstein and Douglas, 1993). ET_B receptors are present on the endothelium and vascular smooth muscle cells. Endothelial ET_B receptors mediate endothelial-dependent vasodilation through the release of NO, prostacyclin and adrenomedullin, while ET_B receptors in vascular smooth muscle cells cause vasoconstriction (Jougasaki et al. 1998; Seo and Lüscher, 1995). However, ET_B receptors in resistance arteries undergo rapid desensitization on exposure to either ET-1 or ET_B selective agonists (Tschudi and Lüscher, 1994). Interestingly, blockade of ET_B receptors, but not ET_A receptors, increases plasma concentrations of ET-1 and ET-3, and prolongs the biological half-life of exogenous [^{125}I]-ET-1 (Löffler et al. 1993; Fukuroda et al. 1994). The increase in ET-1 does not affect concentrations of big ET-1 and C-terminal fragments (Plumpton et al. 1996; Löffler et al. 1993), confirming that the increase is mediated by displacement of ET-1 from receptors rather than through generation. These studies indicate that ET_B receptors play an important role in the clearance of ET in circulation.

Both ET_A and ET_B receptors may be coupled to PLC via a GTP-binding protein (Badr et al. 1989). Activation of PLC causes phosphatidylinositol hydrolysis, rapid formation of IP_3 and DG. IP_3 stimulates the release of Ca^{2+} from intracellular stores. The application of ET-1 to arterial smooth muscle cells causes a rapid transient increase in $[Ca^{2+}]_i$ and a subsequent sustained increase in $[Ca^{2+}]_i$ (Simonson and Dunn, 1990). The

initial transient $[Ca^{2+}]_i$ response is not dependent on the presence of external Ca^{2+} and is the result of IP_3-induced mobilization of Ca^{2+} from intracellular stores. In contrast, the sustained increase of $[Ca^{2+}]_i$ appears to be due to an influx of extracellular Ca^{2+} through either dihydropyridine-sensitive voltage-dependent Ca^{2+} channels (Goto et al. 1989) or receptor-operated cation channels that are insensitive to dihydropyridine (Iwamuro et al. 1998). In addition to the increase in $[Ca^{2+}]_i$, ET receptor activation causes activation of PLA_2 and PLD and changes in arachidonic acid metabolism (Rubanyi and Polokoff, 1994). The activation of PLD appears to contribute to sustained DG accumulation, which may lead to prolonged activation of PKC. In vascular smooth muscle and cardiac muscle, a change in intracellular pH (alkalization) is also induced via stimulation of Na^+ - H^+ exchange, which seems to be a consequence of PKC activation.

In summary, ET isoforms are synthesized from large pre-proforms to pro-peptides or big ETs, and then cleaved by ET converting enzyme (ECE) to the final 21-amino acid peptide. There are two ET receptors, ET_A receptor and ET_B receptors. ET_A and ET_B receptors on vascular smooth muscle cells mediate vasoconstriction, while endothelial ET_B receptors mediate vasodilatation. The main signal transduction pathways of ET-1 are G-protein-mediated activation of PLC, leading to formation of IP_3 and DG, and the subsequent increase in $[Ca^{2+}]_i$ by facilitation of Ca^{2+} influx and mobilization of intracellular Ca^{2+}.

1.1.2.2 Vascular actions of ET-1

Circulating concentrations of ET-like immunoreactivity in venous plasma are in the range 1-10 pmol/L in healthy subjects (Suzuki et al. 1989; Miyauchi et al. 1991).

This immunoreactivity comprises big ET-1 (about 60%), ET-1 (about 30%) and ET-3 (about 10%). ET-2 has not been detected in human plasma. Circulating concentrations of ET-1 are lower than those which cause vascular contraction *in vitro* and *in vivo* . However, cultured endothelial cells secrete substantially more ET-1 towards the adjacent vascular smooth muscle than they do luminally (Yoshimoto et al. 1991). Thus, ET-1 appears to be primarily a locally acting paracrine and/or autocrine substance rather than a circulating endocrine hormone. In addition, ET-1 is rapidly cleared from the circulation after bolus intravenous injection with a biological half life of about 1 min, although its pressor effects are sustained for up to 60 min (Sirvio et al. 1990; Vierhapper et al. 1990). A large proportion of the clearance of ET-1 appears to occur through receptor binding and then internalization.

Intravenous ET-1 infusion produces a transient vasodilatation and hypotensive response, followed by a sustained vasoconstriction and pressor response lasting for at least 60 min. The transient vasodilatation is related to activation of ET_B receptors, while the prolonged vasoconstriction is related largely to activation of ET_A receptors. ET-1 causes sustained contraction of conduit arteries, with a potency 10-fold higher than those of other constrictors (Yanagisawa et al. 1988). The coronary and renal vascular beds in animals are most sensitive to the vasoconstrictor effects of systemic ET-1 (Clozel and Clozel, 1989). The mesenteric bed also vasoconstricts in response to systemic ET-1, whereas the hindquarters skeletal muscle bed exhibits little constriction (Han et al. 1989). These differences among beds may be related to differences in distribution of constrictor (ET_A and ET_B) and dilator receptors (ET_B).

The ETs stimulate generation of NO by vascular endothelial cells (Suzuki et al. 1991; De Nucci et al. 1988).The transient early vasodilator actions of the ETs are attenuated by nitric oxide synthase inhibitors. More relevant physiologically is that NO synthase inhibitors potentiate the constrictor and pressor effects of ET-1, suggesting that there is an autocrine feedback mechanism modulating vasoconstriction in response to ET by stimulation of the endothelial generation of NO. In addition, ET-1 also increases generation of prostacyclin by cultured endothelial cells (Suzuki et al. 1991) and cyclooxygenase inhibitors potentiate ET-1 induced constriction (De Nucci et al. 1988), suggesting that vasodilator prostaglandins play a similar modulator role. However, venoconstriction to ET-1 in humans is potentiated by cyclooxygenase inhibition, but not by NOS inhibition, indicating that prostanoids alone modulate the effects of ET-1 on veins (Haynes and Webb, 1993). These endothelial effects of ET-1 that increase generation of vasodilators appear to be mediated by its ET_B receptors.

The physiological roles of ET-1 in maintenance of vascular tone and BP have been studied by using ET-1 deficient mice and ET-1 antagonists. Mice with one ET-1 gene deleted have slightly higher BP than controls, despite their lower circulating ET-1 (Kurihara et al. 1994). However, there is evidence that the elevation of BP in this model is due to sympatho-adrenal overactivity caused by hypoxia secondary to abnormalities in facial/pharyngeal development (Webb et al. 1998). Now it is believed that the best way to address the physiological role of endogenous ET-1 is to examine the hemodynamic effects of drugs that selectively block the generation or actions of ET-1. Although it is

widely recognized that ET-1 does regulate BP under physiological conditions, the relative contributions of ET_A and ET_B receptors is still a matter of debate.

There is controversy regarding non-selective or selective ET_A antagonists on vascular tone and BP in normotensive animals. Some investigators showed that non-selective (TAK-044) or selective (FR139317 and BQ123) ET_A receptor antagonists have slight but significant hypotensive effects on BP in normotensive subjects or animals (Haynes et al. 1996; Fujita et al. 1995; Bigaud and Pelton, 1992; Pollock et al. 1993), but others could not repeat these results with FR139317 and BQ123 (Sogabe et al. 1993; Bazil et al. 1992). Recently, we reported that both a non-selective ET receptor antagonist, bosentan, and a selective ET_A receptor antagonist, BMS-182874, failed to lower BP in conscious normotensive rats (Balakrishnan et al. 1996; Yu et al. 1998). Although ET does not appear essential to maintain BP in the normotensive animal, one cannot exclude a contributive role of ET because blockade of one control system typically activates other compensatory systems to maintain vascular tone (McNeill, 1983). Indeed, local blockade of forearm resistance vessels with the ET_A receptor antagonist, BQ123, or the non-selective ET receptor antagonist, TAK-044, caused slow-onset forearm vasodilatation (Haynes and Webb, 1994; Haynes et al. 1996), supporting a physiological role of ET-1 in maintenance of vascular tone. Taken together, these data suggest that endogenously generated ET-1 may play a role in maintenance of vascular tone via ET_A receptors.

However, Gellai et al has reported that in contrast to a non-selective ET receptor antagonist or a selective ET_A receptor antagonist, an ET_B receptor antagonist induced

increases in BP and decreases in renal blood flow in normotensive rats (Gellai et al. 1996), indicating that the predominant role of endogenous ET-1 in normotensive animals is vasodilatation via ET_B receptors. Similar hypertensive effects were found with another ET_B receptor antagonist, A192621 (Webb et al. 1998). Thus, under physiological conditions it appears that endogenous ET-1 primarily evokes vasodilation and natriuresis through endothelial and renal ET_B receptors. In addition, intra-arterial infusion of the ET_B receptor antagonist, BQ-788, produced a sustained vasoconstriction in humans and opposed the vasodilator action of BQ-123 (Verhaar et al. 1998). The kidney is rich in ET_B receptors that attenuate tubular reabsorption of sodium and lead to natriuresis (Terada et al. 1992; Kohan and Hughes, 1993; Kohan, 1993). Lack of these renal ET_B receptors in ET_B knockout mice and rats causes sensitivity to salt and hypertension that is not reversible with blockade of ET_A receptors (Webb et al. 1998). The vasoconstrictor effects of ET_B receptor antagonists would be consistent with blockade of endothelial ET_B receptor-mediated formation of NO. However, it is worth remembering that ET_B antagonists increase concentrations of ET-1 by blocking clearance receptors (Löffler et al. 1993; Fukuroda et al. 1994) and this phenomenon could also account for the pressor effects of blockade of ET_B receptors. Indeed, there is evidence that the pressor effects of ET_B receptor antagonism are present even when formation of NO is inhibited and that these effects can be blocked by a ET_A receptor antagonist (Gratton et al. 1997).

In summary, endogenous ET-1 plays a physiological role in control of vascular tone and blood pressure. However, the overall vascular effects depend on the balance of ET_A-mediated and ET_B-mediated effects. Activation of vascular smooth muscle ET_A

receptors causes vasoconstriction and tends to elevate BP, while stimulation of endothelial and renal ET_B receptors promotes vasodilation and natriuresis and tends to decrease BP.

1.1.2.3 Renal actions of ET-1

ET-1 has two main direct actions on the kidney, renal vasoconstriction and natriuresis. ET-1 contracts afferent and efferent arterioles equally and thus reduces both renal plasma flow and glomerular filtration rate (Lopez-Farre et al. 1989; King et al. 1989). In addition, there is substantial production of ET-1 by the inner medullary collecting duct cells (Kohan, 1993), and renal tubular epithelial cells have a high density of ET receptors, mainly of the ET_B subtype (Terada et al. 1992). Several lines of evidence suggest that this locally produced ET-1 plays an important role in modulation of renal excretion of sodium and water. ET-1 inhibits tubular Na^+/K^+-ATPase activity in the proximal tubule and collecting duct to block reabsorption of sodium (Zeidel et al. 1989). Furthermore, ET-1 inhibits the effects of AVP in the collecting duct to block reabsorption of water (Oishi et al. 1991). The cAMP response of inner medullary collecting duct cells to AVP is potentiated in the presence of specific ET-1 antiserum (Kohan and Hughes, 1993), suggesting that endogenous production of ET-1 tonically inhibits responses to AVP. Indeed, ET-1 generation in renal tubule is reduced by an increase in osmolarity *in vitro* and by volume depletion *in vivo* (Kohan and Padilla, 1993; Michel et al. 1993), and the number of ET receptors in glomeruli and tubules of volume-depleted rats is greater than normal (Michel et al. 1993). These tubular effects also occur with ET_B receptor agonists and are not blocked by the ET_A receptor antagonist

BQ-123, suggesting that they are mediated by ET_B receptors (Kohan et al. 1993; Yukimura et al. 1994). The hypothesis that ET_B receptors are involved is also supported by the finding that ET_B knockout mice have hypertension secondary to renal retention of sodium (Webb et al. 1998).

In summary, locally generated ET-1 plays a physiological role in regulating transport of salt and water by inhibiting Na^+/K^+-ATPase activity and the effects of AVP. The ET-1-induced natriuresis and diuresis are activated via ET_B receptors.

1.2 Effects of AVP and ET-1 in DOCA-Salt Hypertensive Rats

Rat models of hypertension can be divided into primary and secondary groups. As reviewed by Pinto et al. (Pinto et al. 1998), primary hypertensive models can be subdivided into genetic, including SHR, Dahl-salt sensitive and transgenic rats, and environmental, including stress-induced hypertensive rats. Secondary hypertensive models can be induced by manipulating endocrine function, such DOCA-salt, or by manipulating renal function, such as 2K1C hypertensive rats. The first animal model of hypertension was developed when Harry Goldblatt clipped the renal artery of a dog (2K1C) and produced a secondary form of hypertension.

Transgenic techniques provide a tool to generate animals that differ from the wild-type, either by introducing foreign genes (transgenic animals) or by specific mutations of genes (knock-out animals). By introducing a mouse Ren-2 gene, the TGR(mRen-2)27 rat is characterized as a renin-angiotensin system dependent hypertensive animal with an inverse circadian blood pressure rhythm and severe end-

organ damage (Witte et al. 1999). However, so far, the SHR is still the most widely used rat model.

DOCA-salt hypertension can be induced by administration of DOCA in combination with a high salt diet and unilateral nephrectomy (Gomez-Sanchez et al. 1996). The model is characterized as a low renin and volume overload form of hypertension. Therefore, it has a different natural history and a different response to antihypertensives, when compared to the high renin models (SHR, 2K1C and TGR(mRen2)27). In fact, this is one of a few hypertensive rat models, in which inhibition of the peripheral renin-angiotensin system does not decrease BP and improve end-organ changes (Aono et al. 1988). However, diuretics (Cabral et al. 1994), ET antagonists (Matsumura et al. 1995) and calcium blockers (Li et al. 1996) are effective both with regard to BP and to end-organ changes. End organ damage in this hypertensive rat model is reported to include cardiac hypertrophy (Matsumura et al. 1995), renal dysfunction (Lafferty et al. 1991) including proteinuria and glomerulosclerosis, and impaired endothelium function (Kirchner et al. 1993).

In addition to AVP (see the following section 1.2.1), the brain renin-angiotensin system has been implicated in DOCA-salt hypertension. In 1986, Itaya et al. reported that intracerebroventricular infusion of captopril, an ACE inhibitor, attenuated the development of hypertension. The prevention of hypertension was accompanied by a reduction in plasma AVP in DOCA-salt hypertension (Itaya et al. 1986). The decrease in plasma AVP was unexpected because the decrease in BP by captopril should have evoked a compensatory increase in AVP levels. This experiment supports the notion that

the brain renin-angiotensin system is coupled to AVP release. A role for the brain renin-angiotensin system in DOCA-salt hypertension is further supported by the fact that Ang II receptor density in specific regions of the brain was increased following DOCA treatment (Gutkind et al. 1988).

Aside from the central renin-angiotensin system, it is likely that the peripheral sympathetic nervous system is involved in the development of DOCA-salt hypertension. This notion is supported by the observation that central catecholaminergic depletion attenuated this form of hypertension (Lamprecht et al. 1977), and that plasma NE and catecholamine level were higher in DOCA-salt treated rats than in normotensive rats (Bouvier and Champlain, 1986).

1.2.1 AVP in DOCA-salt hypertension

A primary role for AVP in the pathogenesis of DOCA-salt hypertension was first suggested by Friedman *et al.* (Friedman et al. 1960). He found that administration of AVP accelerated the development of hypertension in rats treated with DOCA, and that ablation of the median eminence prevented the development of hypertension. In DOCA-salt hypertensive rats, plasma and urine AVP levels were reported to be increased (Mohring et al. 1977; Crofton et al. 1979), and the severity of hypertension was found to be related to the plasma level of AVP. However, AVP binding sites in mesenteric vascular bed were significantly reduced due to the increases in plasma concentration (Lariviere et al. 1988). Administration of non-selective or selective V_2 receptor antagonists significantly attenuated the development of hypertension (Okada et al. 1995) and lowered BP in the established phase of the DOCA-salt hypertension (Hofbauer et al.

1984; Galatius-Jensen et al. 1996; Okada et al. 1995). Compelling evidence for a role of AVP in the DOCA-salt model arose from studies in Brattleboro (DI) rats homozygous for diabetes insipidus, which lack the ability to synthesize the hormone. These DI rats failed to develop hypertension when treated with DOCA and salt, but did develop hypertension after co-treatment with AVP or dDAVP, a V_2 receptor agonist (Crofton et al. 1979; Satio and Yajima, 1982).

However, the role of V_1 receptors in DOCA-salt hypertensive rats is controversial. Pressor responsiveness to AVP has been reported to be increased (Matsuguchi and Schmid, 1982; Mimura et al. 1995), unchanged (Burnier et al. 1983), or decreased (Filep et al. 1985) in DOCA-salt hypertensive rats compared to its normotensive control. Administration of V_1 receptor antagonists produced small decreases (Hiwatari et al. 1986; Burrell et al. 1994; Intengan et al. 1998) or no change in BP (Filep et al. 1987; Toba et al. 1994; Okada et al. 1995). Failure to induce any change in BP does not preclude a role of the V_1 receptor in this hypertensive animal model because blockade of one system typically activates other systems to regulate BP (McNeill et al. 1977; McNeill, 1983). Due to the contribution of the ET system in the DOCA-salt model (see the following section 1.2.2), it was postulated that there may be a redundant or reciprocal relationship between the AVP and ET systems in maintaining the high BP and vascular tone observed in this model. *Therefore, a part of this thesis project was to study the role of V_1 receptors in the maintenance of hemodynamics in DOCA-salt hypertensive rats both when the ET system was functional and when it was prevented from compensating.*

In summary, there is compelling evidence that circulating AVP is essential for the development and maintenance of the hypertensive state in the DOCA-salt hypertensive model, although the pressor role of AVP in this model is controversial.

1.2.2 ET-1 in DOCA-salt hypertension

Several lines of evidence support a role of ET-1 in the development and maintenance of hypertension in DOCA-salt hypertensive rats. First, immunoreactive ET-1 content is increased in aorta and mesenteric arteries from DOCA-salt hypertensive rats (Lariviere et al. 1993b; Fujita et al. 1995), but not in those from SHR. Second, preproET-1 gene expression in aorta and mesenteric arteries is also increased in DOCA-salt hypertensive animals (Lariviere et al. 1993a), suggesting the increased ET-1 content is secondary to the increased preproET-1 gene expression. Third, in heart, ET-1 mRNA expression and immunoreactive ET-1 concentration in ventricles from DOCA-salt hypertensive rats are elevated (Lariviere et al. 1995). Fourth, both non-selective ET_A/ET_B and selective ET_A receptor antagonists have been reported to attenuate the development of hypertension and to decrease BP in established hypertension in this model (Li et al. 1994; Yu et al. 1998; Fujita et al. 1995; Bird et al. 1995). Fifth, vascular hypertrophy and remodeling seen in DOCA-salt hypertensive rats are attenuated with chronic treatment of a non-selective ET antagonist (Li et al. 1994). However, ET-1 production does not seem to contribute to the cardiac hypertrophy seen in this hypertensive animal, since the increased level of ET-1 mRNA transcripts was found exclusively in endothelial cells of blood vessels and endocardium, but not in myocardial

cells (Lariviere et al. 1995). In conclusion, all of these observations suggest a role of ET-1 in the pathogenesis of hypertension in the DOCA-salt hypertensive model.

Changes in BP with non-selective or selective ET_A receptor antagonists in DOCA-salt hypertensive rats might be due to changes in CO or in total peripheral resistance (TPR), and the relative contribution of these two variables to changes in BP induced by ET antagonists have not been reported. *Therefore, an objective of this thesis was to address this issue directly by monitoring BP with radiotelemetry devices and CO with ultrasonic transit-time flowprobes in conscious DOCA-salt hypertensive rats during administration of the non peptide mixed ET receptor antagonist, bosentan, and the selective ET_A receptor antagonist, BMS-182874.*

In summary, there is convincing evidence supporting a role of ET-1 in the pathogenesis of hypertension in the DOCA-salt hypertensive model, and this involvement of ET appears to be mediated at least in part via ET_A receptors.

1.2.3 Interactions between AVP and ET-1

1.2.3.1 ET on AVP secretion

ET has been documented to increase AVP and ANP release peripherally and centrally. Intravenous administration of ET induced a significant elevation of BP and plasma levels of AVP, ANP and renin activity, and decreases in urine flow and Na excretion in dogs (Miller et al. 1989). In addition, ET was also found to be present in the central nervous system, including the hypothalamo-neurohypophysial structures (Takahashi et al. 1991; Nakamura et al. 1993). Moreover, ET mRNA and ET receptors

were distributed within brain sites implicated in the central control of cardiovascular function and AVP release. Intracerebroventricular infusion of ET-1 at a dose of 3.5 ng/kg/min evoked increases in BP and plasma levels of AVP and ANP, and decreases in urine flow and urinary Na excretion in conscious rats (Yamamoto et al. 1992). These increases in BP and plasma ANP, and the decrease in urine flow could be attenuated by the peripheral pretreatment of a V_1 receptor antagonist, suggesting that the increased release of AVP contributes to ET central effects. Similarly, Rossi et al. reported that icv injection of ET-1 in rats caused increases in BP and plasma levels of AVP (Rossi et al. 1997). However, it was the enhanced sympathetic outflow, but not AVP, that contributed to the changes in BP elicited by ET centrally. Regardless of the mechanism of BP elevation, ET does increase AVP release by its action peripherally and centrally.

1.2.3.2 AVP on ET secretion

Many endogenous physical and chemical stimuli have been reported to stimulate the synthesis and release of ET-1 from endothelial cells (see section 1.1.2.1). Among them, AVP and Ang II have been reported to stimulate ET-1 secretion from cultured bovine carotid artery endothelial cells (Emori et al. 1991) and human mesangial cells (Bakris et al. 1991; Bakris and Re, 1993). The increased release of ET-1 is due mainly to the increased expression of preproET-1 mRNA stimulated by AVP and Ang II (Imai et al. 1992). In perfused mesenteric vascular beds, AVP was showed to increase ET-1 release (Tomobe et al. 1993b). Infusion of AVP, but not Ang II, has been reported to increase plasma ET-1 levels in conscious dogs (Emmeluth and Bie, 1992). In tissue studies, Chen et al. from our lab reported that, in contrast to rat aorta, Ang II-induced

contractions of rat mesenteric and tail arteries were endothelium dependent and could be partially or completely attenuated by an ET_A receptor antagonist in normotensive rats (Chen et al. 1995b). Similarly, responses of the rabbit aorta to Ang II were attenuated by BQ123, an ET_A receptor antagonist (Webb et al. 1992). In SHR, Dohi et al. found that the contraction of the rat mesenteric artery evoked by NE was potentiated by Ang II. This potentiation was endothelium dependent and was prevented by pre-treatment of the tissue with phosphoramidon, an ET converting enzyme inhibitor, or an ET antibody (Dohi et al. 1992), indicating the involvement of ET in the Ang II-induced potentiation of NE responses. In DOCA-salt hypertensive rats, Intengan et al. showed that a V_1 receptor antagonist attenuated the enhanced preproET-1 gene expression in mesenteric resistance arteries (Intengan et al. 1998), suggesting ET-1 may be involved in part in mediating the vascular effects of AVP in DOCA-salt hypertension.

Although considerable evidence exists at the cellular and tissue levels, there has been little effort to quantify the contribution of ET-1 to responses evoked by AVP and Ang II at the hemodynamic level. Recently from our lab, Balakrishnan et al. demonstrated that hemodynamic responses to low doses of AVP (1, 3 and 10 ng/kg/min) or to low doses of Ang II (3 and 9 ng/kg/min) were blunted by a non peptide mixed ET receptor antagonist in SHR, a genetic model of hypertension with high renin levels (Balakrishnan et al. 1997; Balakrishnan et al. 1996), indicating the involvement of ET in hemodynamic responses to AVP and Ang II in this genetic animal model. However, the contribution of ET-1 to AVP and Ang II at the hemodynamic level in the DOCA-salt hypertensive rat, an experimental hypertensive animal model with low renin level has not

been reported. In view of the involvement of both AVP and ET in the development and maintenance of hypertension in this experimental hypertensive animal and in view of their interaction *in vitro* and *in vivo*, it is reasonable to postulate that an ET component contributes to the hemodynamic effects of AVP in DOCA-salt hypertensive rats. *Thus, a major goal of this thesis project was to quantify the contribution of ET-1 to the changes in BP, CO and TPR induced by AVP in conscious DOCA-salt hypertensive rats. By recording both flow and pressure, it was possible to calculate TPR and thus determine if any contribution was at the level of factors regulating CO, or at the level of factors regulating the resistance function of the circulation. For comparison, the responses to Ang II were also evaluated in this model. A final objective was to determine if any ET component was different in hypertensive and normotensive rats.*

1.3 Hypothesis and predictions of the working hypotheses

1.3.1 Hypothesis

ET and AVP contribute to the maintenance of hypertension in DOCA-salt hypertensive rats by constricting resistance vessels. Part of the increase in resistance evoked by AVP in this hypertensive animal model is due to an ET-1 component.

1.3.2 Working hypotheses and predictions

I. *ET contributes to the maintenance of hypertension in DOCA-salt hypertensive rats via ET_A receptor-induced vasoconstriction of resistance vessels.* A prediction of this hypothesis is that a non-selective or selective ET_A receptor antagonist should lower

TPR and BP in this hypertensive animal model to a similar extent. To test this prediction, the relative contributions of CO and TPR to changes in BP were recorded before and after bosentan, a non-selective ET antagonist, or BMS-182874, a selective ET_A receptor antagonist.

II. *The contribution of either AVP or ET to the maintenance of BP and vascular tone may be underestimated when one system is allowed to compensate.* A prediction of this hypothesis is that the magnitude of the response to an ET receptor antagonist will be greater in rats pretreated with a V_1 receptor antagonist and conversely, the response to a V_1 receptor antagonist will be greater in rats pretreated with an ET receptor antagonist. To test this prediction, changes in BP, CO and TPR were recorded during the administration of [$d(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-Vasopressin, a V_1 receptor antagonist, before and after the treatment with bosentan. In other experiments, bosentan was administered before and during the administration of [$d(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-Vasopressin.

III. *ET contributes to the hemodynamic effects of AVP in the DOCA-salt hypertensive rat.* A prediction of this hypothesis is that the hemodynamic effects of AVP should be attenuated by the blockade of ET receptors in DOCA-salt hypertensive rats. To test this prediction systematically, changes in BP, CO and TPR were monitored during administration of AVP before and after bosentan.

2. Materials and Methods

2.1 General

2.1.1 Animals

All experiments and protocols were performed in accordance with the regulations established by the Canadian Council on Animal Care and approved by the animal care committee at the University of Saskatchewan. Male Sprague-Dawley (SD) rats were purchased from Charles River (St. Constant, Quebec) at 6 wk of age and raised in our animal quarters under standardized conditions. Some SD rats were first or second generation animals bred and raised in our animal quarters. In our final data analysis, there was no difference in responses among these rats.

Rats were selected at 8-10 weeks of age, at which time the right kidney was removed through a dorsal flank incision under ether anesthetization. One week later, these rats were divided randomly into two groups, DOCA- and SHAM-groups. In the DOCA-group, a Silastic strip impregnated with 100 mg/kg body wt of DOCA was implanted subcutaneously in the midscapular region. From this point on, these rats were given a 0.9% NaCl-0.2% KCl solution for drinking *ad libitum* over a 3-week period. Under this regimen, this group of rats developed hypertension. In the SHAM-controlled group, a DOCA-free Silastic strip was implanted subcutaneously. Tap water was provided as a drinking solution over the next 3 weeks. The Silastic strip was made in a

plastic groove model from a mixture of medical grade elastomer, silastic curing agent (10:1 w/w) with or without DOCA (10 mg/cm).

2.1.2 Chemicals

The chemicals used in the experiments and their sources are listed as following:

AVP [(Arg^8)-Vasopressin] from Bachem (Torrance, CA);

Ang II (octapeptide) from Bachem;

ET-1 from American Peptide Co. (Sunnyvale, CA);

[$d(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-Vasopressin, a V_1 receptor antagonist, from Bachem;

Sodium pentobarbital from MTC pharmaceuticals;

Deoxycorticosterone Acetate (DOCA) from Sigma (St. Louis, MO), Aldrich Chem. Co. (Milw., WI), ICN Biomedicals Inc.(Aurora, OH);

0.9% sodium chloride (saline) from ABBOTT;

Medical grade elastomer and silastic curing agent from DOW CORNING (Midland, MI);

BMS-182874 provided by Bristol Myers Squibb, NJ;

Bosentan provided by Dr. M. Clozel (F. Hoffman-La Roche, Basel).

Stock solutions of AVP (100 μg/ml), [$d(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-vasopressin (100 μg/ml), Ang II (500 μg/ml) and ET-1 (25 μg/ml) were made with 0.2M acetic acid and stored at -80 or -20 degree C. Working solutions were then made on the day of the experiment by diluting the stock solution with 0.9% saline. Solutions of ET-1 were prepared in 1% bovine serum albumin (BSA). BMS-182874 and bosentan were dissolved in 5% $NaHCO_3$ and sterile water respectively.

2.1.3 Equipment

Radiotelemetry system

The components of the radiotelemetry system consisted of the following: 1) a 10-cm catheter with a 1 cm tip constructed of thin walled tubing which was coated with an anti-thrombogenic film. The tip of the catheter had been pre-filled with a viscous gel to prevent blood from entering the catheter; 2) an implantable capsule (TA11PA-C40, Data Sciences, St. Paul, MN) which houses a highly stable semiconductor strain gauge sensor and a battery powered electronic module to process the information from the pressure sensor and transmit it from animals to receivers; 3) a receiver (RLA 1020) which detects the signal from the implanted transmitter and transmits it to a data acquisition system; 4) a barometric pressure reference (C11PR) which measures the atmospheric pressure to allow for the telemetered absolute pressure (relative to a vacuum) to be converted to a gauge pressure (relative to atmospheric pressure); 5) a BCM100 consolidation matrix which multiplexes the signals from a number of receivers and provides power to the telemetry receivers; 6) a data acquisition system (DATAQUEST IV) which accepts data from the reference and the receiver, filters corrupt samples from the incoming data stream, converts the telemetered pressure to mmHg, subtracts atmospheric pressure from the telemetered pressure and stores the data for further retrieving, plotting and analyzing. The catheter material provides for easy insertion, excellent kink resistance and minimal vessel irritation. The technique of radiotelemetry system for measuring BP has been validated in our lab and by others (Balakrishnan et al. 1998; Bazil et al. 1993).

Transonic system

The Transonic system for small animal blood flow measurement consists of a bench top electronic flowmeter (T206, Transonic Systems Inc., Ithaca, NY) and a volume flow sensing probe. Using wide beam illumination, two transducers pass ultrasonic signals back and forth, intersecting the flowing liquid in upstream and downstream directions. The flowmeter derives an accurate measure of the 'transit time' it takes for the ultrasound wave to travel from one transducer to another. The difference between the upstream and downstream integrated transit times is a measure of volume flow. The flowprobes can sense volume flow independent of flow velocity. The 2.5 SB series flow probe was used for recording CO in these experiments. The signal from the flowmeter was fed into a pen recorder (model 7P03, Grass Instrument Co. Quincy, MA).

Others

Model 7 polygraph, Model 7DAF DC driver amplifier from Grass Instument Co.;

Model 975 infusion pump from Harvard;

Model 941 infusion/withdrawal pump from Harvard;

Model 683 small animal ventilator from Harvard;

Model K-550-G vortex from Scientific Industries Inc.

2.1.4 Miscellaneous

Micro-renathane tubing: MRE 040 from Braintree Scientific;

TEFLON tubing (0.59 mm ID x 0.83 mm OD and 0.47 mm ID x 0.71 mm OD) from B-D;

Orthocryl powder and liquid from Stratford-Cookson Company;

Dacron mesh from Small Parts Inc.;

Silk sutures 4-0 from Ethicon.

2.2 Surgical procedures

After 3-week of a DOCA or DOCA-free treatment, the animals were anesthetized with Somnotol, (sodium pentobarbital, 50 mg/kg) peritoneally. Endotracheal intubation was performed and the rat was attached to the ventilator. All the following surgeries were performed under aseptic conditions. At least a 10-day recovery period was provided following the surgery before the animals were used for experimentation.

2.2.1 Implantation of the Transonic flowprobe

A median sternotomy was performed, and a microretractor was used to expose the thymus and heart. After the pericardium was opened, the ascending aorta was gently separated from the pulmonary artery by gentle dissection using micro-dissecting forceps. The J-reflector of a 2.5SB series ultrasonic flow probe (zero offset $\leq \pm 3$ ml/min, absolute accuracy $\pm 15\%$), which was used to measure cardiac output (CO), was implanted around the ascending aorta and a small piece of surgical absorbable hemostat was placed between the J-reflector and the right-anterior vena cava. Then, the chest was closed by using 4-0 sutures and the animal was weaned from the respirator. The flowprobe cable was tunneled subcutaneously and the connector was exited at the back of the neck.

2.2.2 Implantation of the radiotelemetric device

Before implantation of the radiotelemetry capsule, the zero of each transducer was verified to be = ± 4 mmHg. The catheter of a telemetry device was inserted into the

left femoral artery and advanced so that the tip of the catheter was in the abdominal aorta above the iliac bifurcation. After experiments, some rats were sacrificed to verify the position of the tip. The tip of the catheter was in abdominal aorta and below the renal arteries. The capsule containing the transducer and radiotransmitter was positioned in the left flank region subcutaneously.

2.2.3 Cannulation of the femoral vein

The left and right femoral veins were cannulated with MRE040 tubing, which had been filled with 0.9% NaCl. The free ends of the catheters were tunneled subcutaneously and emerged at the back of neck. The ends of the tubing were then connected to a chronic cannula fashioned with a L-shaped 23G connector embedded in dacron mesh with dental cement. The cannula was fixed subcutaneously and plugged with a 23G obturator. These catheters were used for infusions.

2.3 Experimental protocols

2.3.1 General

10 days after surgeries, these rats regained their body weights from the loss of surgery. BP and CO were recorded in conscious, unrestrained rats. Individual rat cages were placed on receivers (RLA 1020, Data Science) for recording of BP. Pressure data were collected with a computer driven data acquisition system (Dataquest IV, Data Sciences). The pressure waveform was sampled every 30 seconds with a 5-second sample duration. CO was recorded by feeding the signal from the flowmeter (T206

Transonic Systems Inc.) to a pen recorder (Grass Instrument Co. Quincy, MA). Mean CO was recorded continuously. TPR was calculated as the quotient of BP and CO. Two days before the recording, these rats were conditioned in the recording room. On the day of the experiment, a 2-hour control period was given before any drug infusion.

2.3.2 ET dependent component in DOCA-salt hypertension

2.3.2.1 Responses to a non-selective ET antagonist

After a 2-hour control period, a bolus dose of bosentan (30 mg/kg) or vehicle (distilled water) was injected into the femoral vein of both DOCA-salt hypertensive rats and SHAM-rats. These rats were recorded for another 3 hours continuously. The bosentan infused DOCA-salt hypertensive rats were then recorded 24-hour later to monitor the time course of the recovery. The dose of bosentan used here had been tested to produce a maximum inhibition of ET-1 induced pressor responses (Balakrishnan et al. 1996; Clozel et al. 1994). Bosentan was dissolved in distilled water just before each experiment.

2.3.2.2 Responses to a selective ET_A antagonist

After a 2-hour control period, a bolus dose of BMS-182874 (100 μmol/kg) or vehicle (5% $NaHCO_3$) was injected into the femoral vein of both DOCA-salt hypertensive rats and SHAM-rats. These rats were recorded for another 3 hours continuously. The BMS-182874 infused DOCA-salt hypertensive rats were then recorded for the next 3 consecutive days to monitor the time course of the recovery. The

dose of BMS-182874 used here has been tested to produce a maximum inhibition of ET-1 induced pressor response (Webb et al. 1995; Bird et al. 1995).

2.3.3 AVP dependent component in DOCA-salt hypertension

2.3.3.1 Effectiveness of bosentan

To affirm bosentan, a mixed non-peptide ET antagonist, used in this study was an active and competitive antagonist at 100 minutes after its injection, the relationship between doses of ET-1 and responses elicited by this peptide in the presence and absence of bosentan was studied. Bosentan (30 mg/kg) or vehicle (distilled water) was infused intravenously in DOCA-salt hypertensive rats (n=6) and SHAM-control rats (n=6). 100-min later, bolus doses of ET-1 at 0.03, 0.06, 0.1 and 0.3 nmol/kg were injected intravenously in a cumulative fashion every 10 minutes. The responses to ET-1 in the presence and absence of bosentan in each animal were separated by 3 days in a cross-over design.

2.3.3.2 Effectiveness of [$d(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-Vasopressin

To affirm [$d(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-Vasopressin, a V_1 receptor antagonist, used in this study was active and a competitive antagonist of AVP, the relationship between doses of AVP and hemodynamic responses in the presence or absence of the V_1 receptor antagonist was studied. [$d(CH_2)_5^1$, O-Me_Tyr^2,Arg^8]-Vasopressin (8 μg/kg followed by 0.05 μg/kg/min) or saline was infused intravenously in DOCA- and SHAM-rats. 30-min later, AVP at doses of 1, 3, 10 and 30 ng/kg/min was infused through

another femoral vein catheter for 10 min. in a cumulative graded fashion every 10 min.. These responses to AVP in the presence and absence of the V_1 receptor antagonist in each animal were separated by a 2-day interval in a randomized cross-over fashion.

In section 2.3.3.1 and 2.3.3.2 studies, DOCA-salt hypertensive rats were treated with DOCA and salt for 2 weeks before surgical intervention, instead of 3-week's DOCA and salt pretreatment in the rest of the experiments reported in this thesis.

2.3.3.3 Sequential antagonism of ET and AVP

After a 2-hr control period, a single dose of bosentan (30 mg/kg) was injected intravenously to both DOCA- and SHAM-rats. 100-min later, a bolus dose of 8 μg/kg of [$d(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-vasopressin was injected and was followed by a 0.05 μg/kg/min infusion. The 100-min period after bosentan was chosen because the response to bosentan had reached a plateau by this time (see section 3.1.2 and Fig. 1). The response to the V_1 receptor antagonist was recorded for another 30 min.

In another group of animals, the sequence of the ET and AVP antagonists was reversed. After a 2-hour control period, the V_1 receptor antagonist (8 μg/kg followed by 0.05 μg/kg/min) was administered intravenously to DOCA- and SHAM- rats. Thirty minutes later, a bolus dose of bosentan (30 mg/kg) was injected through another venous catheter. Responses were recorded for another 100 min.

2.3.4 ET dependent component of AVP in DOCA-salt hypertension

2.3.4.1 Responses to AVP

One hundred minutes after an intravenous injection of bosentan (30 mg/kg) or vehicle, single doses of [Arg^8]-vasopressin (AVP) were infused intravenously at rates of 1, 3, 10 or 30 ng/kg/min for 15 min. Only one of these four doses of AVP was selected for each rat, and 6-10 DOCA- or SHAM-rats were used for each dose. The selection of the dose was randomized and the responses to AVP in the presence or absence of bosentan were separated by a 4-day interval in a cross-over design. The 100-min period after bosentan was chosen because the response to bosentan in DOCA-rats had reached the plateau by this time.

2.3.4.2 Responses to Ang II

This protocol was identical to that for AVP, except that Ang II was infused at rates of 3, 9, 30 or 90 ng/kg/min for 10-min.

2.4 Data analysis

All values are expressed as mean ● SEM. Graphs of the residuals were plotted to detect heterogeneity of variances. If needed, homogeneity of variances was achieved by log transformation of the data. P values of less than 0.05 were considered statistically significant.

2.4.1 ET dependent component in DOCA-salt hypertension

Control values in both DOCA- and SHAM-groups were compared by analysis of variances (ANOVA) based on contrasts. Changes in BP, CO and TPR to bosentan or BMS-182874 and vehicle were performed by two-factor ANOVA (time and treatment).

2.4.2 AVP pressor component in DOCA-salt hypertension

Control values in both strains and effects of different treatments were compared by repeated-measurements of one (treatment or time) or two factors (time and treatment) ANOVA.

2.4.3 ET dependent component of AVP in DOCA-salt hypertension

Responses to each dose of AVP in the presence or absence of bosentan within the DOCA- or SHAM-group was compared by ANOVA for repeated measurement. Responses to AVP between these two groups was analyzed by one-factor ANOVA.

3. Results

3.1 ET dependent component in DOCA-salt hypertension

3.1.1 Control values

Table 1 and 2 show the control values for BP, CO and TPR in DOCA-salt hypertensive rats and SHAM-rats treated with bosentan and BMS-182874 respectively. BP was significantly higher in DOCA-salt hypertensive rats than in SHAM-rats. The elevated BP appeared to be due to elevated systemic resistance, as TPR was significantly increased in DOCA-salt hypertensive rats. The values for CO were not significantly different in the two groups.

3.1.2. Responses to bosentan

The time-course of the changes of BP, CO, and TPR evoked by bosentan are depicted in Figures 1, 2, and 3. In DOCA-salt hypertensive rats, bosentan significantly decreased BP (Fig. 1) and TPR (Fig. 3) and increased CO (Fig. 2) compared to the pre-infusion control values and to the vehicle control values. Significant depressor effects were observed at 30 min and the fall in pressure reached a plateau between 90 – 180 minutes. Twenty-four hours later, all variables had returned towards control values. In contrast to the DOCA-salt group, the hemodynamic changes in the SHAM-group were

unremarkable (Fig. 1, 2 and 3).

Table 1. Control values in the bosentan treatment group

	DOCA-rats	***SHAM-rats***
	n=16	*n=10*
BP (mmHg)	141±2*	103±3
CO (ml/min/kg)	167±10	178±11
TPR (mmHg/ml/min/kg)	0.89±0.05*	0.60±0.04

Values (means ± sem) for blood pressure (BP), cardiac output (CO) and total peripheral resistance (TPR) in SHAM-operated rats (SHAM-rats) and DOCA-salt hypertensive rats (DOCA-rats) before bosentan treatment (3 determinations in each rat). *P<0.05 compared to SHAM-rats.

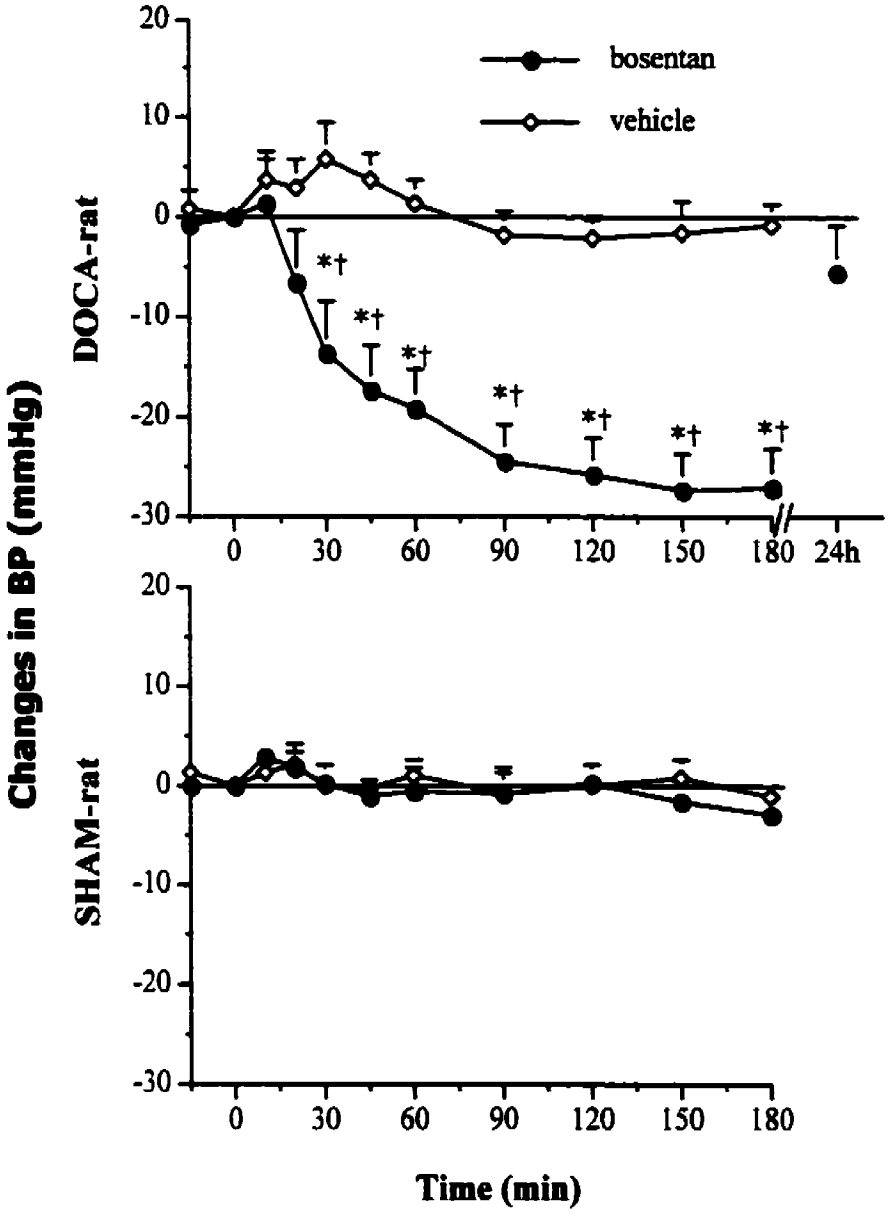

Figure 1. Changes in blood pressure (BP) in DOCA-salt hypertensive rats (DOCA-rat) and sham-control rats (SHAM-rat) evoked by i.v. injections of bosentan and vehicle at 0 time. *P<0.05 compared to 0 values, †P<0.05 compared to vehicle controls.

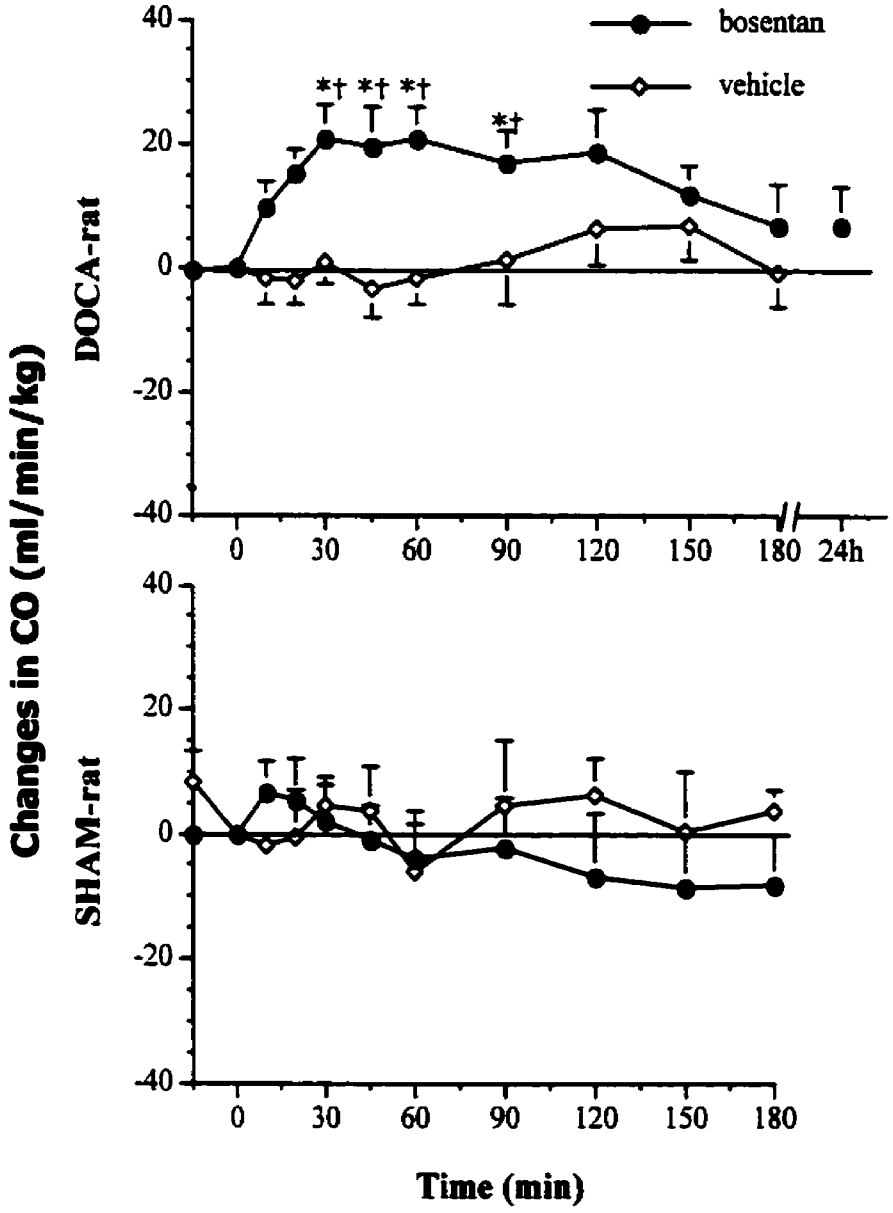

Figure 2. Changes in cardiac output (CO) in DOCA-salt hypertensive rats (DOCA-rat) and sham-control rats (SHAM-rat) evoked by i.v. injections of bosentan and vehicle at 0 time. *P<0.05 compared to 0 controls, †P<0.05 compared to vehicle controls.

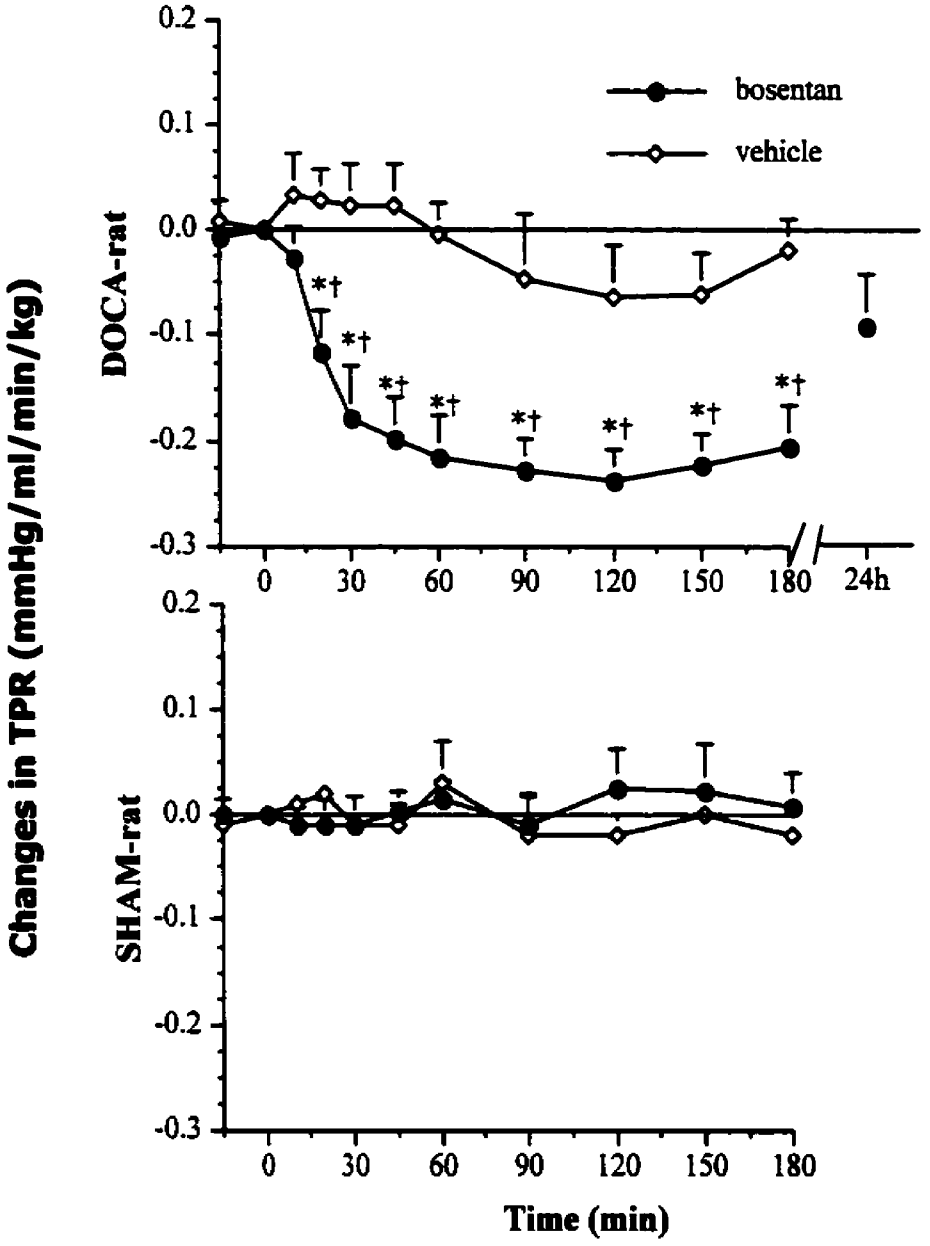

Figure 3. Changes in total peripheral resistance (TPR) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat) evoked by i.v. injections of bosentan and vehicle at 0 time. *P<0.05 compared to 0 values, †P<0.05 compared to vehicle controls.

3.1.3 Responses to BMS-182874

The changes of BP, CO and TPR are showed in Figure 4, 5 and 6. In DOCA-salt hypertensive rats, BMS-182874 significantly decreased BP (Fig. 4) and TPR (Fig. 6) compared to pre-infusion control values and to vehicle controls. The significant hypotensive effect of BMS-182874 started at 45 min after its infusion and lasted more than 24 hours. CO was elevated compared to vehicle control but this effect of the ET antagonist was small (Fig 5). The changes in BP, CO, and TPR returned to control values within 48-72 hours. In contrast to the DOCA-salt group, the hemodynamic changes in the SHAM-group were unremarkable (Fig. 4, 5 and 6).

Table 2. Control values in the BMS-182874 treatment group

	DOCA-rats	***SHAM-rats***
	n=12	n=10
BP (mmHg)	140±3*	105±2
CO (ml/min/kg)	181±5	197±5
TPR (mmHg/ml/min/kg)	0.78±0.03*	0.54±0.02

Values (means ± sem) for blood pressure (BP), cardiac output (CO) and total peripheral resistance (TPR) in SHAM-operated rats (SHAM-rats) and DOCA-salt hypertensive rats (DOCA-rats) before BMS-182874 treatment (3 determinations in each rat). *P<0.05 compared to SHAM-rats.

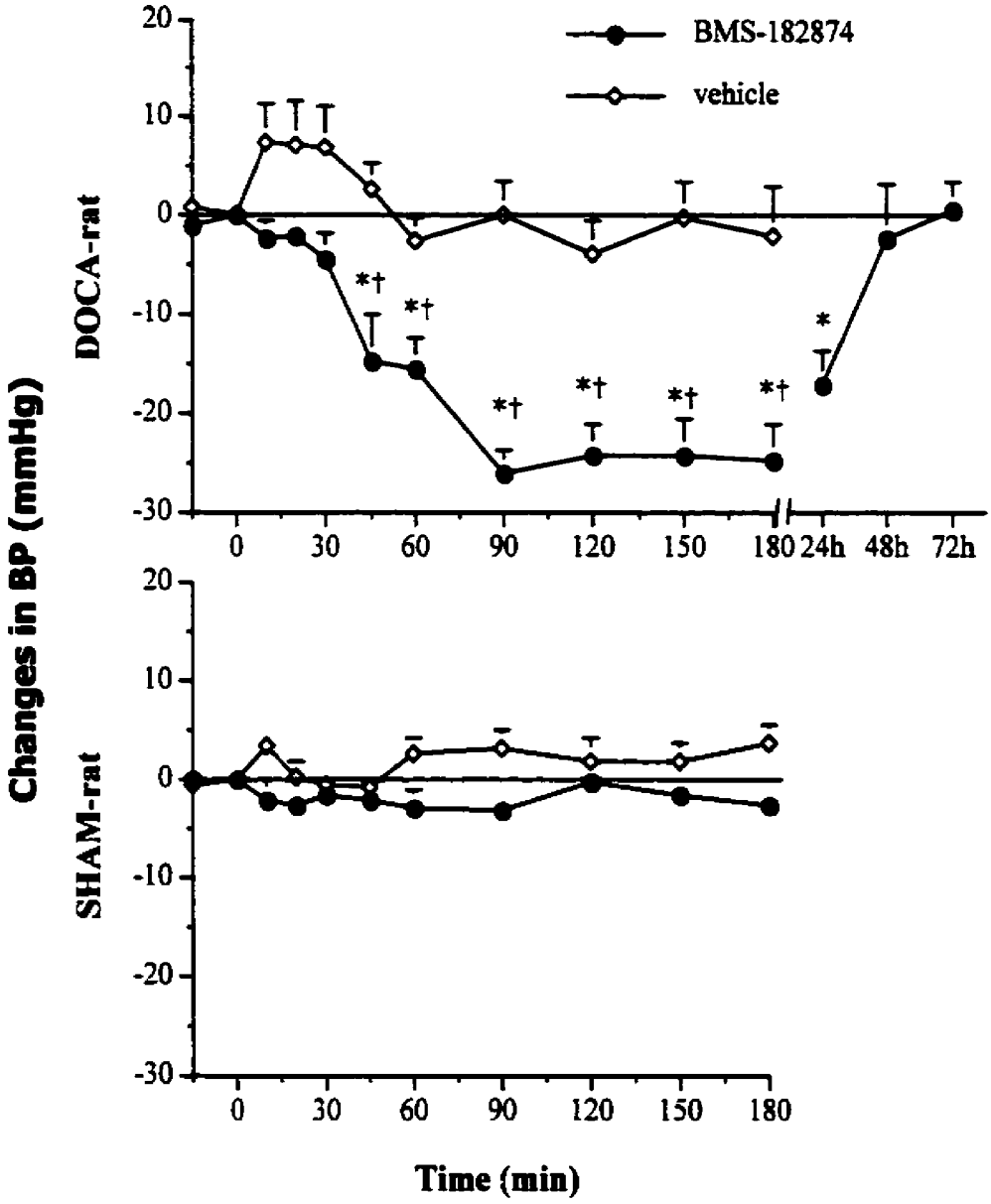

Figure 4. Changes in blood pressure (BP) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat) evoked by i.v. injections of BMS-182874 and vehicle at 0 time. *P<0.05 compared to 0 value, †P<0.05 compared to vehicle control.

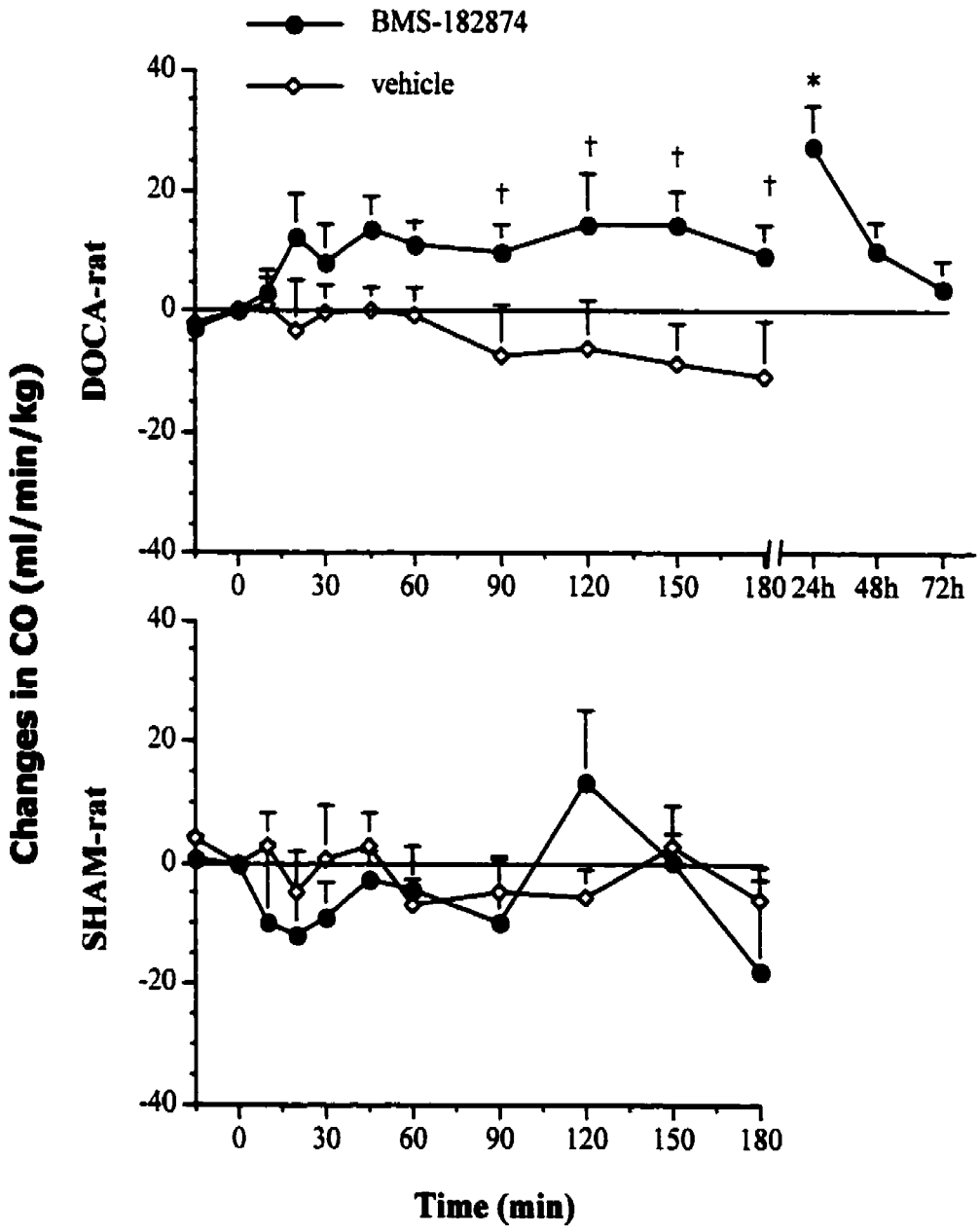

Figure 5. Changes in cardiac output (CO) in DOCA-salt hypertensive rats (DOCA-rat) and sham-control rats (SHAM-rat) evoked by i.v. injections of BMS-182874 and vehicle at 0 time. *P<0.05 compared to 0 values, †P<0.05 compared to vehicle controls.

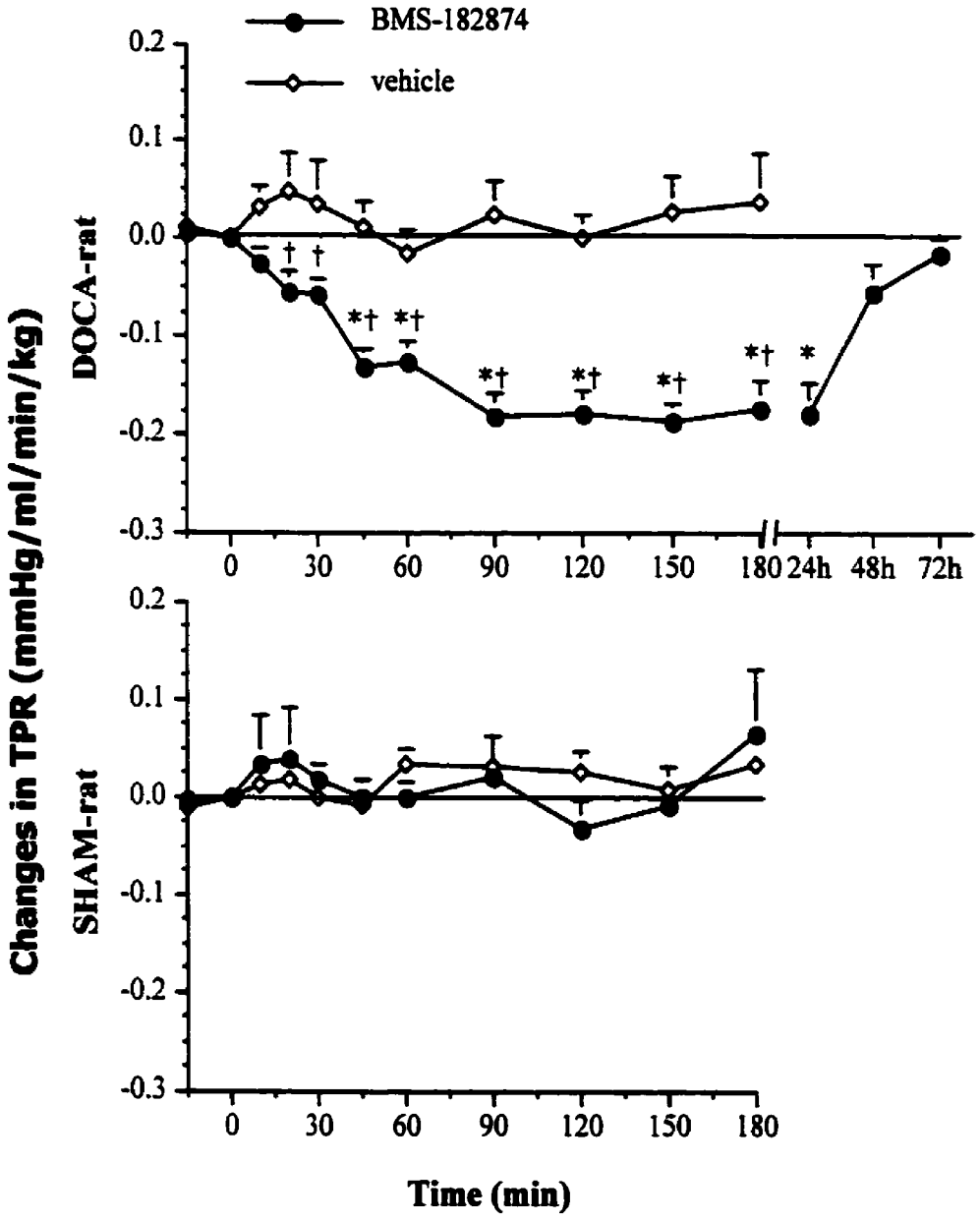

Figure 6. Changes in total peripheral resistance (TPR) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat) evoked by i.v. injections of BMS-182874 and vehicle at 0 time. *P<0.05 compared to 0 values, †P<0.05 compared to vehicle controls.

3.2 AVP dependent component in DOCA-salt hypertension

3.2.1 Effectiveness of bosentan

3.2.1.1 Control values and effects of bosentan

Control values of BP, CO and TPR in DOCA- and SHAM- rats are shown in Table 3. Before bosentan, BP was significantly higher in DOCA-salt hypertensive rats than in SHAM-control rats. This elevated BP appeared to be due to the increased TPR, as the values for CO were not significantly different. In contrast to SHAM-control rats, bosentan lowered BP in DOCA-salt hypertensive rats at 100-min after its administration.

3.2.1.2 Responses to ET-1

The changes in BP, CO and TPR in DOCA-salt hypertensive rats and SHAM-control rats to cumulative i.v. injections of ET-1 in the presence or absence of bosentan are depicted in Fig. 7, 8 and 9. ET-1 induced dose-related increases in BP and TPR, and the vasoconstriction was associated with a decrease in CO. The pressor responses induced by lower doses of ET-1 were exaggerated in DOCA-rats compared to SHAM-rats (Fig. 7), and the elevated pressor effect was due mainly to the significantly increased vascular resistance, i.e., increased TPR (Fig. 9). Bosentan pretreatment attenuated the changes in BP, CO and TPR to ET-1 in both groups of rats.

Table 3. Control values for effectiveness of bosentan

	DOCA-rats n=6		*SHAM-rats* n=5	
Parameter	Control	Bosentan	Control	Bosentan
BP (mmHg)	136±3*†	114±6	103±2	104±3
CO (ml/min/kg)	188±5	205±7	200±10	213±12
TPR (mmHg/ml/min/kg)	0.73±0.02*†	0.56±0.04	0.54±0.04	0.49±0.02

Values (means ± sem) for blood pressure (BP), cardiac output (CO) and total peripheral resistance (TPR) in SHAM-operated rats (SHAM-rats) and DOCA-salt hypertensive rats (DOCA-rats) before and 100-min after bosentan treatment. *P<0.05 compared to SHAM-rats and †P<0.05 compared to bosentan treatment.

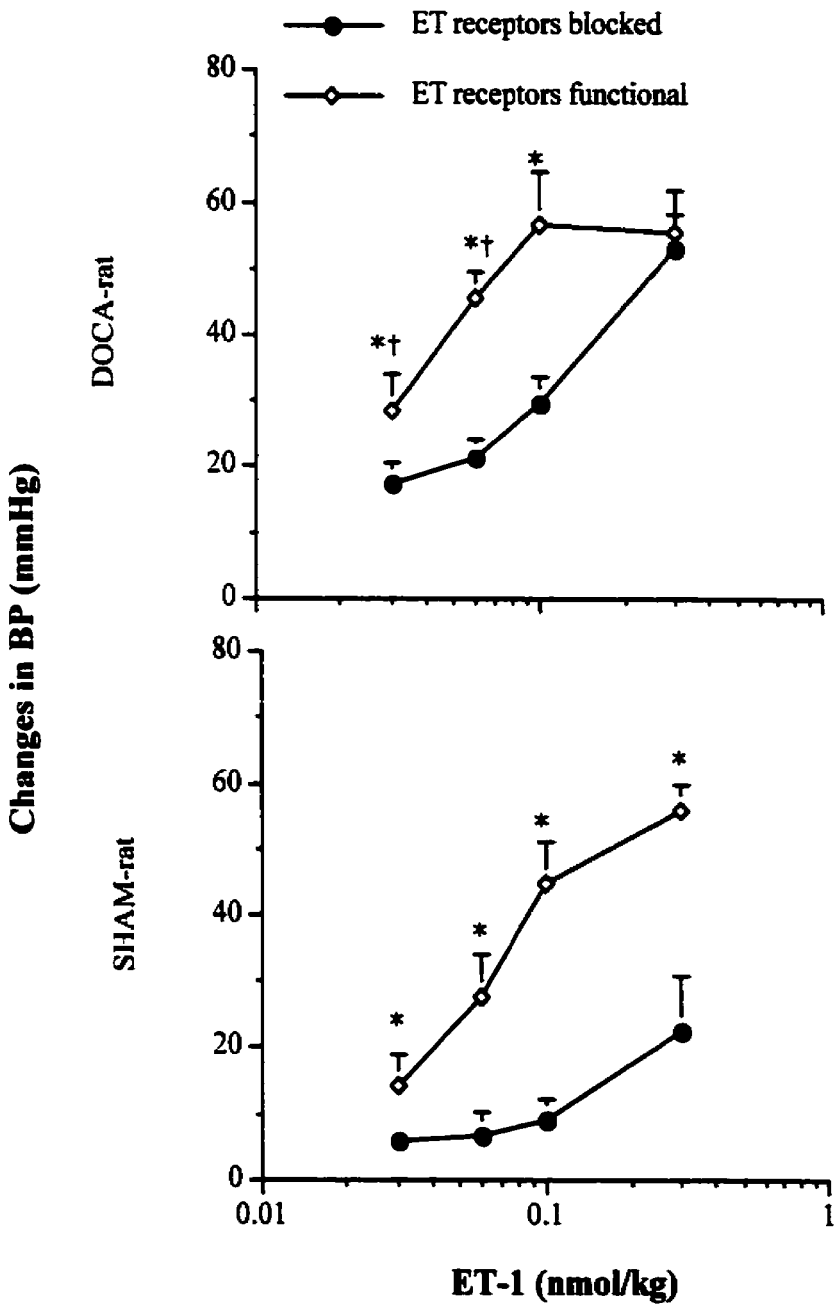

Figure 7. Changes in blood pressure (BP) to cumulative i.v. injections of ET-1 before (ET-receptors functional) and after bosentan pretreatment (ET-receptors blocked) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat). *P<0.05 compared to ET-1 with bosentan. †P<0.05 compared to SHAM-rats.

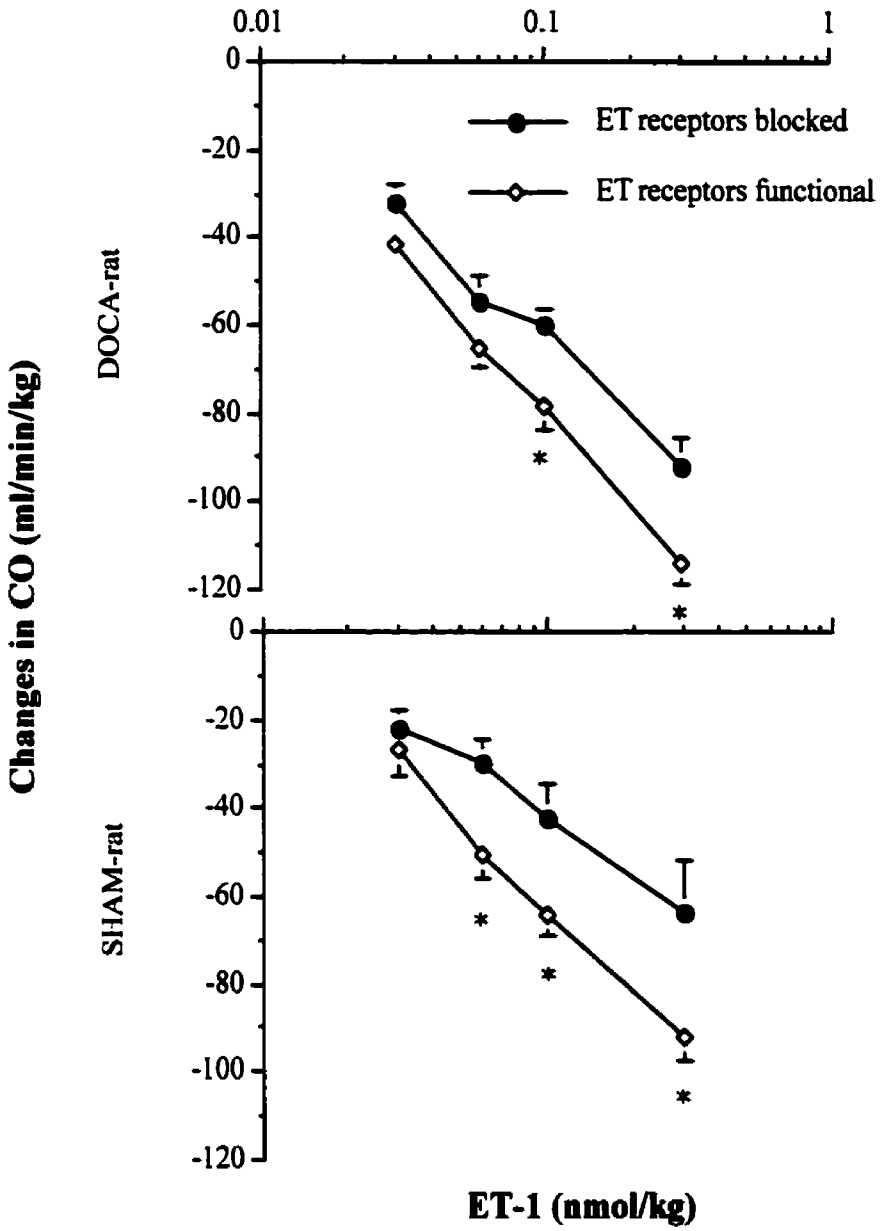

Figure 8. Changes in cardiac output (CO) to cumulative i.v. injections of ET-1 before (ET-receptors functional) and after bosentan pretreatment (ET-receptors blocked) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat). *P<0.05 compared to ET-receptors blockade.

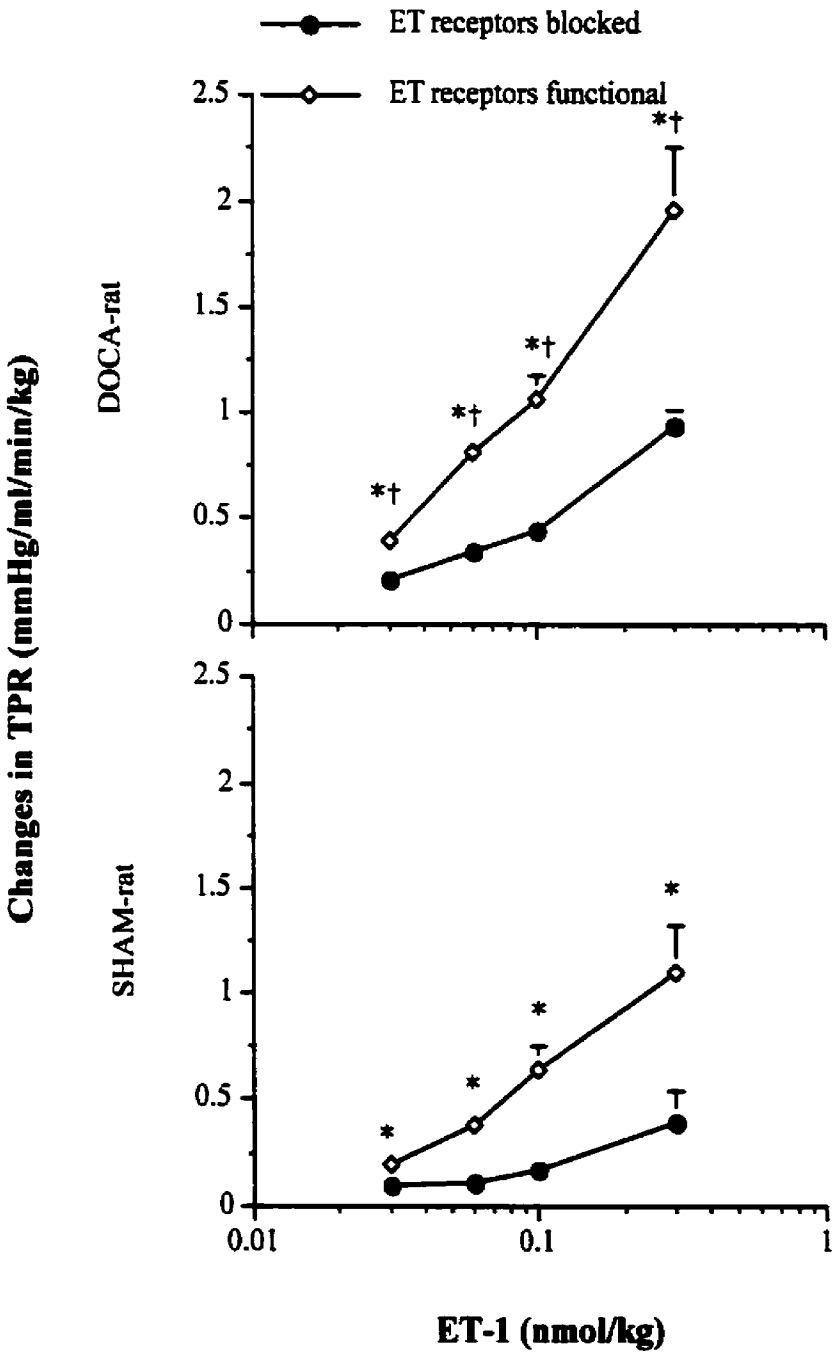

Figure 9. Changes in total peripheral resistance (TPR) to cumulative i.v. injections of ET-1 before (ET-receptors functional) and after bosentan pretreatment (ET-receptors blocked) in DOCA-salt hypertensive rats (DOCA-rat) and sham-control rats (SHAM-rat). *P<0.05 compared to ET-receptors blockade and †P<0.05 compared to SHAM-rat.

3.2.2 Effectiveness of [d(CH_2)$_5^1$, O-Me-Tyr^2,Arg^8]-Vasopressin

3.2.2.1 Control values and effects of the V_1 antagonist

Control values of BP, CO and TPR in DOCA- and SHAM- were shown in Table 4. Before the V_1 receptor antagonist treatment, BP was significantly higher in DOCA-salt hypertensive rats than in SHAM-control rats. This elevated BP appeared to be due to the increased TPR, as the values for CO were not significantly different. The V_1 receptor antagonist failed to induce significant hemodynamic changes in both groups.

3.2.2.2 Responses to AVP

Changes in BP, CO and TPR in DOCA-salt hypertensive and SHAM-control rats to cumulative i.v. infusions of AVP in the presence or absence of the V_1 receptor antagonist are depicted in Fig. 10, 11 and 12. AVP evoked dose-dependent increases in BP and TPR, and decreases in CO in both strains. AVP failed to exert exaggerated pressor responses in DOCA-rats compared to SHAM-rats (Fig. 10). However, the increase in TPR to the lowest dose of AVP in DOCA-rats was significantly higher than in SHAM-rats (Fig. 12). Pretreatment with the V_1 receptor antagonist abolished these changes in BP, CO and TPR evoked by AVP.

Table 4. Control values for effectiveness of [d$(CH_2)_5^1$, O-Me-Tyr^2,Arg^8]-Vasopressin

	DOCA-rats n=6		***SHAM-rats*** n=5	
Parameter	Control	V_1 ant.	Control	V_1 ant.
BP (mmHg)	135±6*	136±6	111±2	111±4
CO (ml/min/kg)	197±13	195±11	218±26	218±28
TPR (mmHg/ml/min/kg)	0.70±0.06*	0.71±0.05	0.55±0.08	0.54±0.08

Values (means ± sem) for blood pressure (BP), cardiac output (CO) and total peripheral resistance (TPR) in SHAM-operated rats (SHAM-rats) and DOCA-salt hypertensive rats (DOCA-rats) before and 30-min after the V_1 receptor antagonist (V_1 ant.) treatment.
*P<0.05 compared to SHAM-rats.

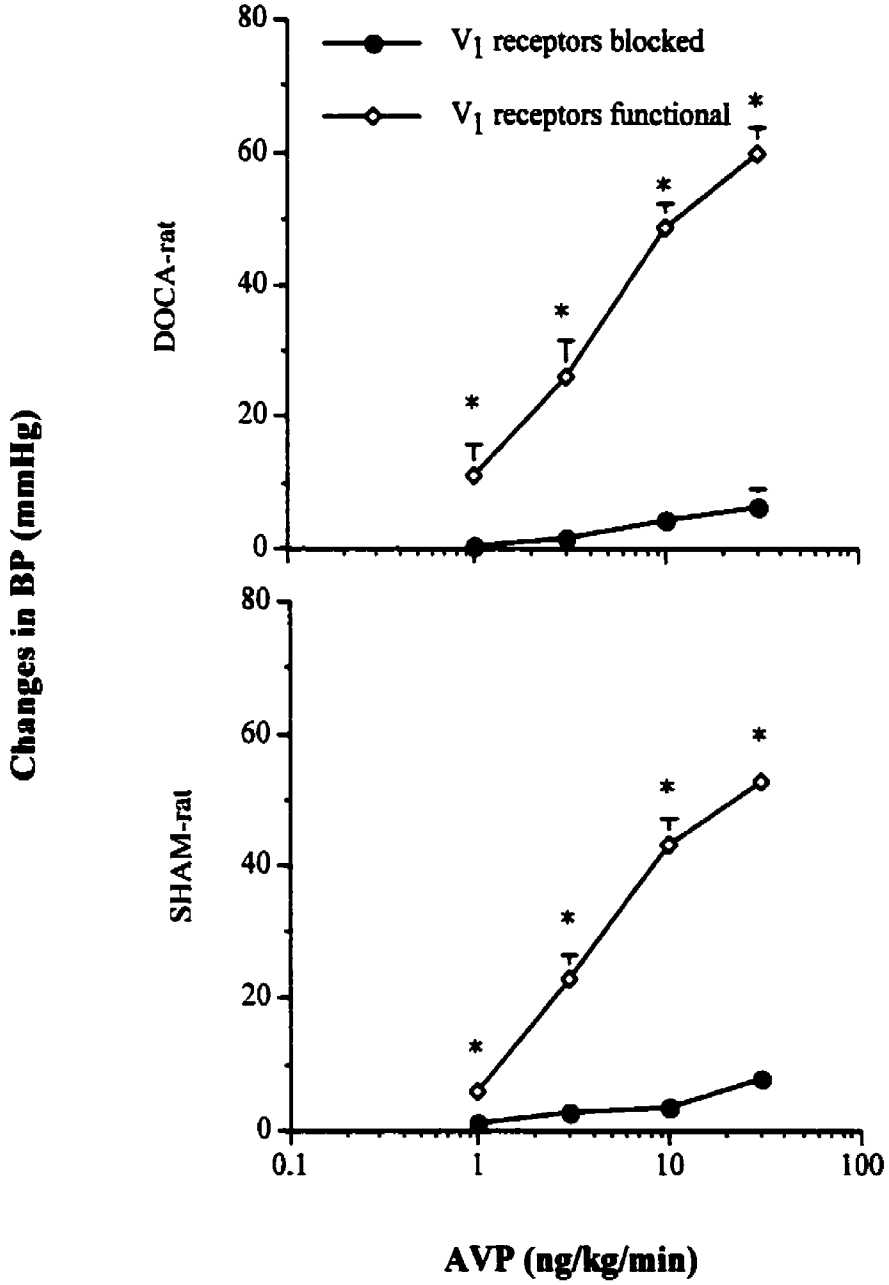

Figure 10. Changes of blood pressure (BP) to cumulative i.v. infusions of AVP without (V_1 receptors functional) and with the V_1 antagonist pretreatment (V_1 receptors blocked) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat). *P<0.05 compared to the V_1 antagonist pretreatment group.

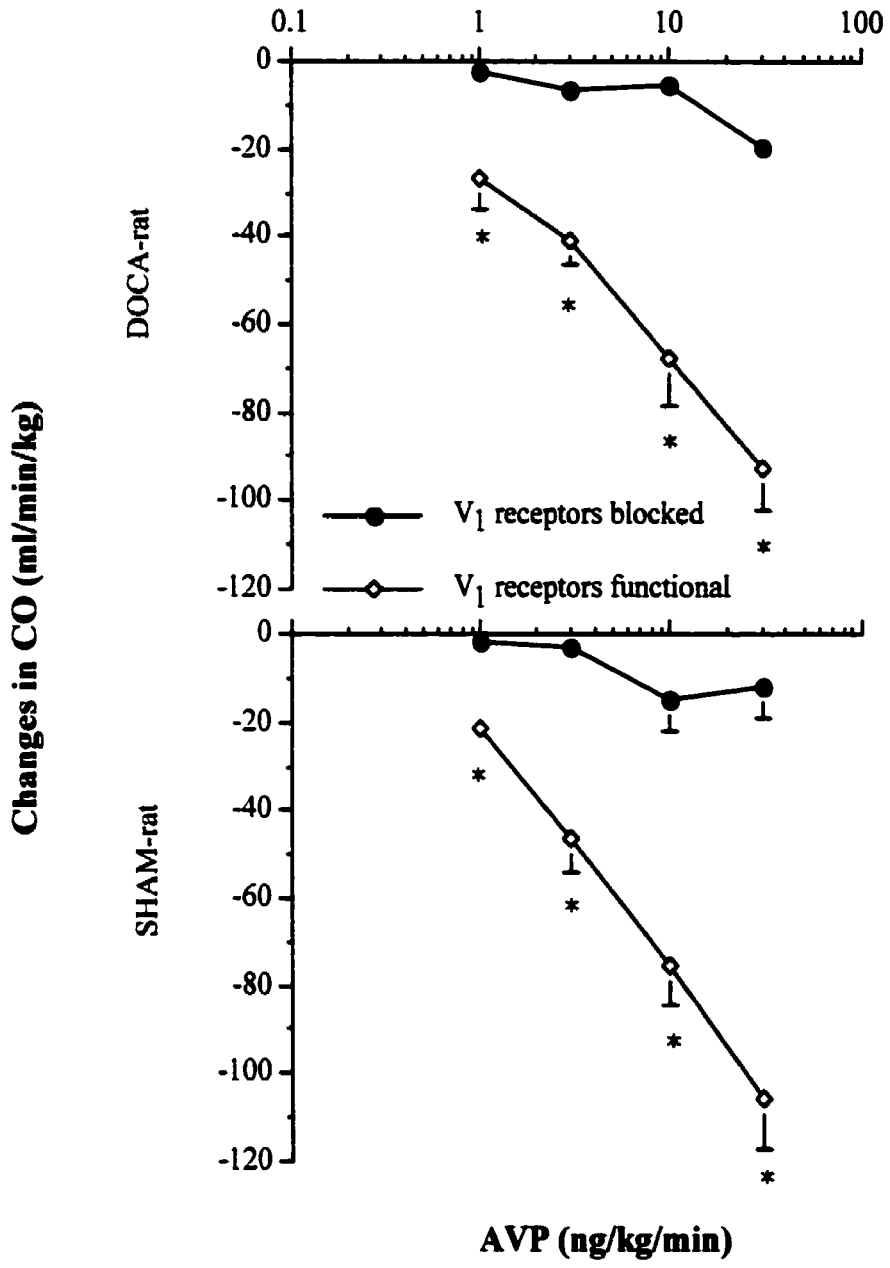

Figure 11. Changes in cardiac output (CO) to cumulative i.v. infusions of AVP without (V_1 receptors functional) and with the V_1 antagonist pretreatment (V_1 receptors blocked) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat). *P<0.05 compared to the V_1 antagonist pretreatment group.

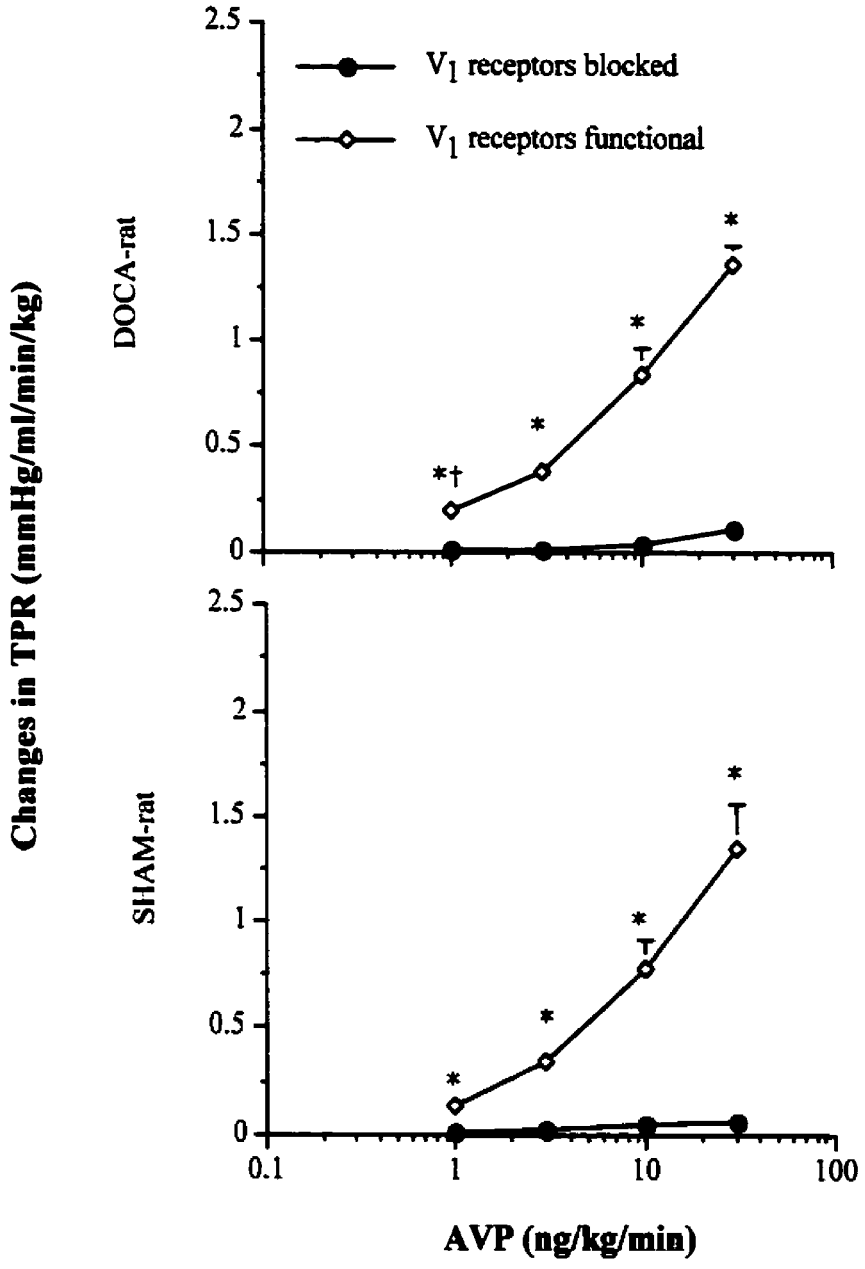

Figure 12. Changes of total peripheral resistance (TPR) to cumulative i.v. infusions of AVP without (V_1 receptors functional) and with the V_1 antagonist pretreatment (V1 receptors blocked) in DOCA-salt hypertensive rats (DOCA-rat) and sham control rats (SHAM-rat). *P<0.05 compared to the V_1 antagonist pretreatment group and †P<0.05 compared to SHAM-rats.

3.2.3 Reciprocal compensatory roles of AVP and ET-1

3.2.3.1 Control values

Control values of BP, CO and TPR in DOCA-salt hypertensive and SHAM- rats in this study are shown in Table 5. BP was significantly higher in DOCA-rats than in SHAM rats. This elevated BP appeared to be due to the increase in TPR, not to the factors regulating CO.

3.2.3.2 Roles of AVP and ET

The time-course of the changes in BP, CO and TPR in DOCA-salt hypertensive and SHAM- control rats treated with bosentan followed by the V_1 receptor antagonist are shown in Fig. 13. In contrast to SHAM-control rats, bosentan significantly decreased BP and TPR in DOCA-salt hypertensive rats. After the blockade of ET receptors, the V_1 antagonist lowered BP and TPR below the levels achieved by bosentan alone: the values recorded during the 15-min infusion of the V_1 receptor antagonist were significantly lower than those recording at the 100-min mark. In SHAM control rats, the combination of the two antagonists lowered BP and TPR significantly, but these changes were small. The changes in CO were unremarkable.

The time-course of the changes in BP, CO and TPR in DOCA-salt hypertensive and SHAM-rats treated with the V_1 receptor antagonist followed by bosentan are depicted in Fig. 14. In the first 30-min period, the V_1 antagonist alone did not elicit significant changes in BP, CO and TPR in either DOCA-salt hypertensive rats or

SHAM-control rats. After the blockade of V_1 receptors, bosentan significantly lowered BP and TPR compared to the 0-min control values and to its preinfusion values (at the 30-min mark) in DOCA-salt hypertensive rats. In SHAM-rats, the combination of the two antagonists lowered BP significantly but only at the 130-min mark and the change was small.

Comparison of effects of the V_1 receptor antagonist with and without blockade of the ET system in DOCA-salt hypertensive and SHAM rats are depicted in Fig. 15 and 16. The V_1 receptor antagonist alone failed to induce significant changes in BP and TPR. However, with the pretreatment of bosentan, the V_1 receptor antagonist decreased BP and TPR significantly compared to its preinfusion values in DOCA-salt hypertensive rats.

The effect of bosentan with and without blockade of V_1 receptors in DOCA-salt hypertensive and SHAM rats are showed in Fig. 17 and 18. Even in the absence of the V_1 receptor antagonist, bosentan lowered BP and TPR in the DOCA-salt hypertensive rats. However, the magnitude of these changes induced by bosentan was much greater when the rats were pretreated with the V_1 receptor antagonist.

Table 5. Control values in bosentan and the V1 antagonist treatment study

	DOCA-rats	***SHAM-rats***
	n=16	n=13
BP (mmHg)	155±4*	109±3
CO (ml/min/kg)	186±8	185±10
TPR (mmHg/ml/min/kg)	0.86±0.05*	0.61±0.03

Values (means ± sem) for blood pressure (BP), cardiac output (CO) and total peripheral resistance (TPR) in SHAM-operated rats (SHAM-rats) and DOCA-salt hypertensive rats (DOCA-rats). *P<0.05 compared to SHAM-rats.

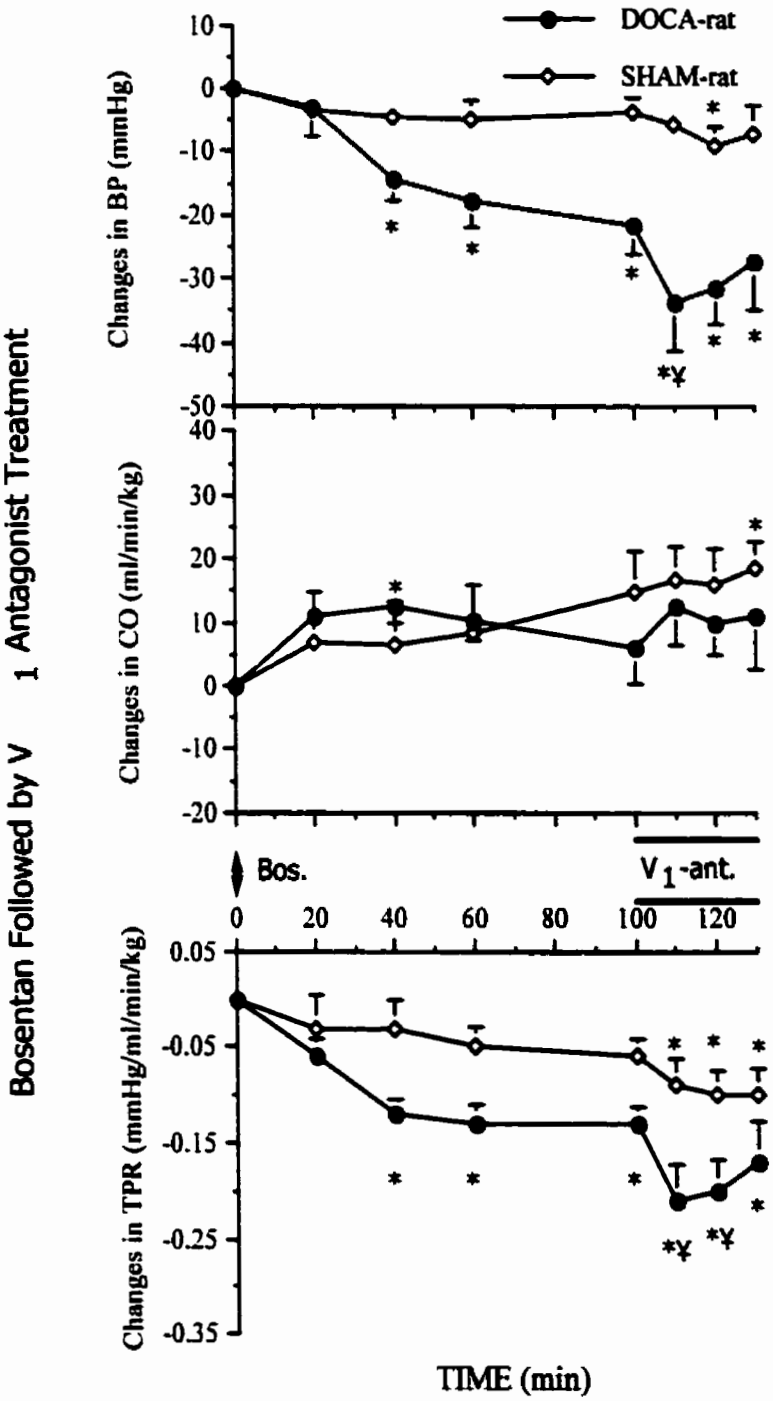

Figure 13. Time-course of the changes in BP, CO and TPR of DOCA-salt hypertensive and SHAM rats treated with bosentan (Bos.) followed by the V_1 antagonist (V_1-ant.). *p<0.05 compared to 0-min values; ¥p<0.05 compared to values at the start of the V_1-ant.(ie 100-min).

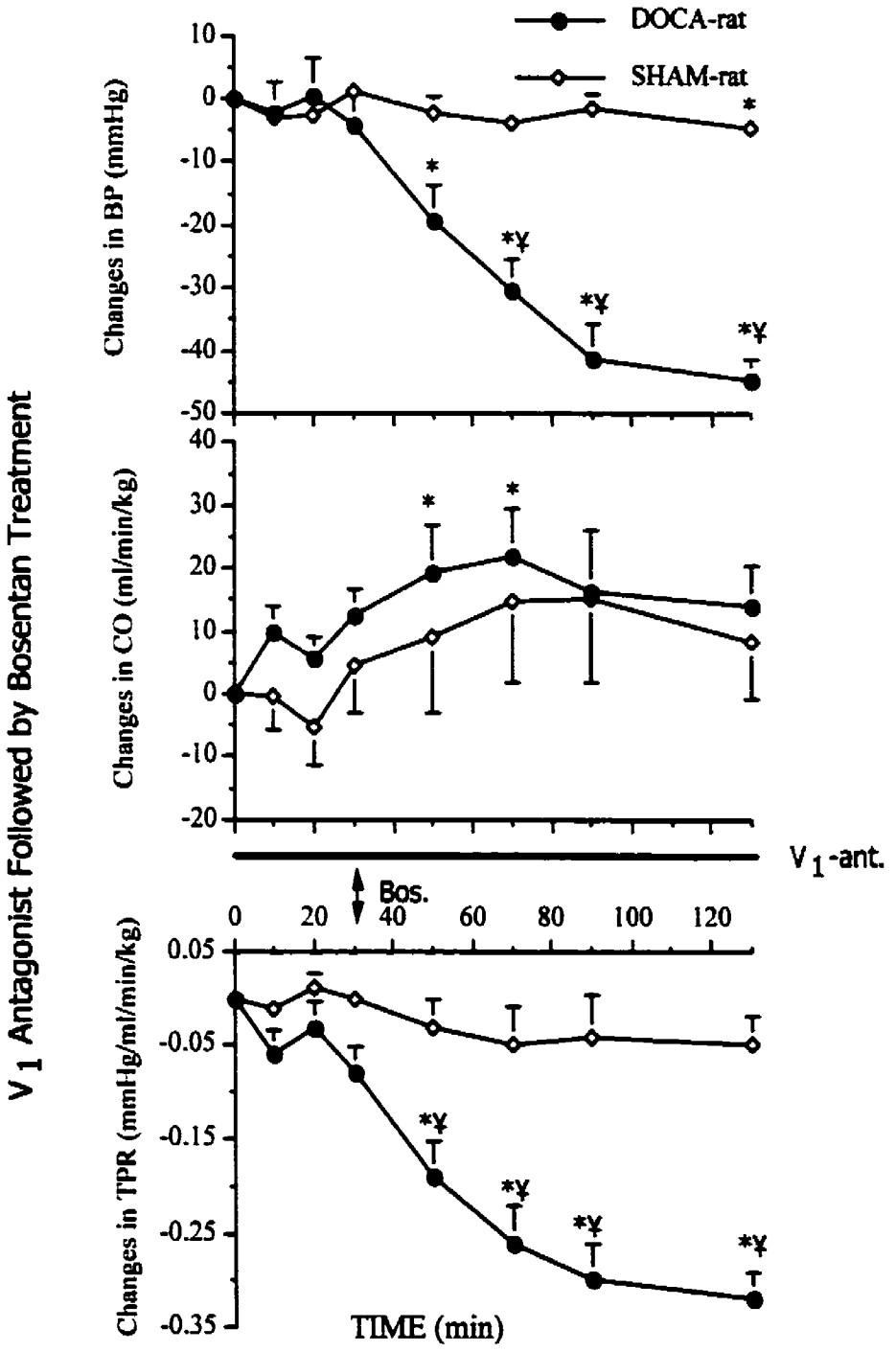

Figure 14. Time-course of the changes in BP, CO and TPR of DOCA-salt hypertensive and SHAM rats treated with the V_1 antagonist (V_1-ant.) followed by bosentan (Bos.). *p<0.05 compared to 0-min values. ¥P<0.05 compared to values at the start of Bos. (ie. 30-min).

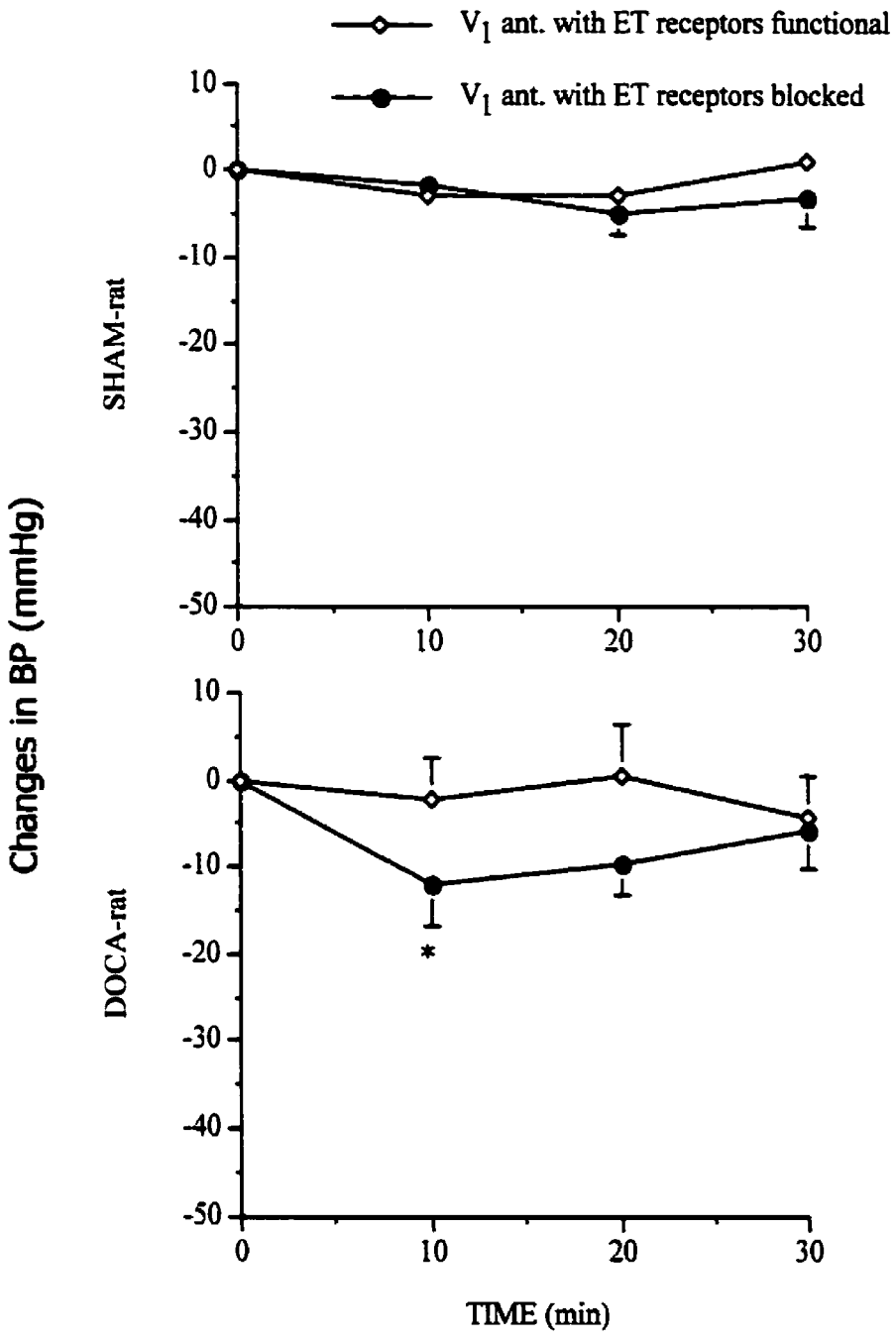

Figure 15. Changes in blood pressure (BP) in SHAM- or DOCA-salt hypertensive rats treated with the V_1 antagonist with (V_1 ant. with ET receptors blocked) and without bosentan (V_1 ant. with ET receptors functional) pretreatment. *P<0.05 compare to the initial value.

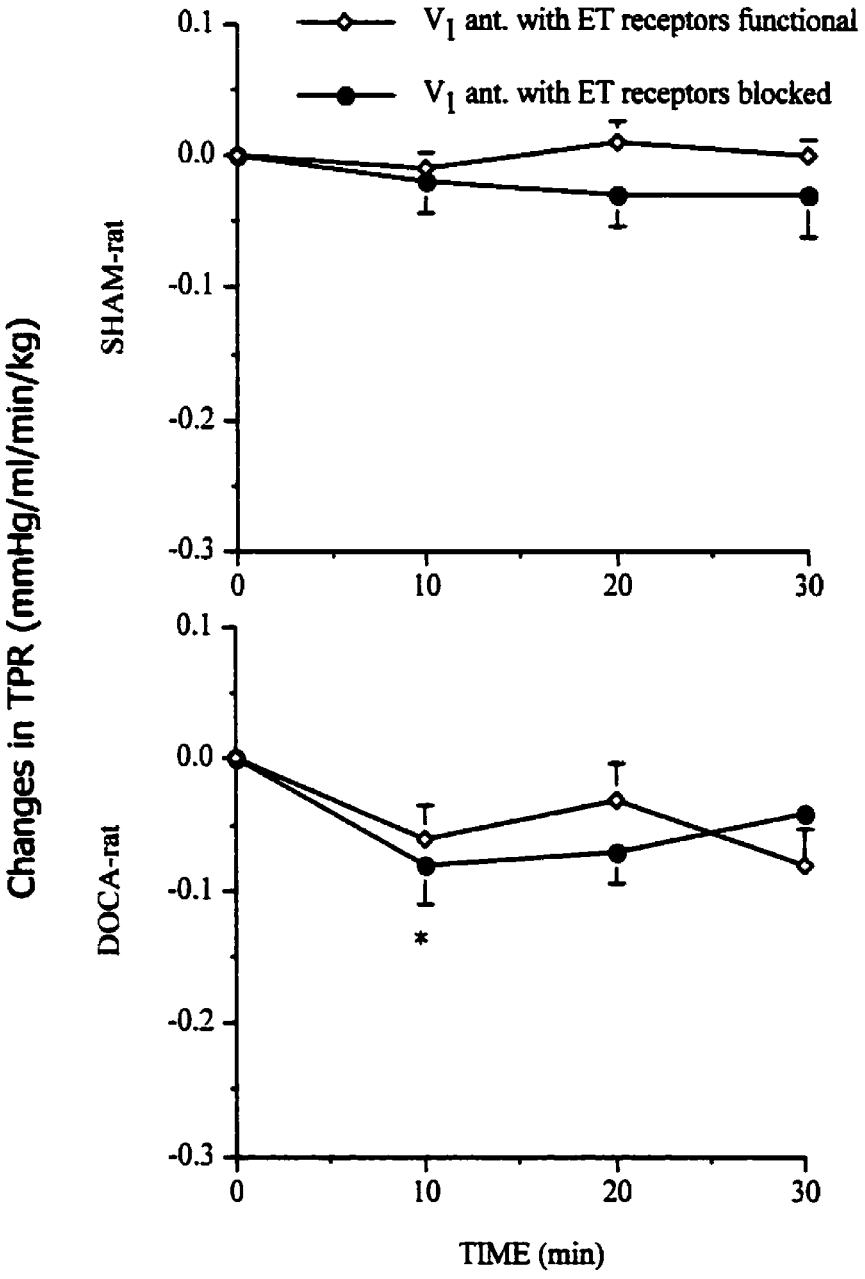

Figure 16. Changes in total peripheral resistance (TPR) in SHAM- or DOCA-salt hypertensive rats treated with the V_1 antagonist with (V_1 ant. with ET receptors blocked) and without bosentan (V_1 ant. with ET receptors functional) pretreatment. *P<0.05 compare to the initial value.

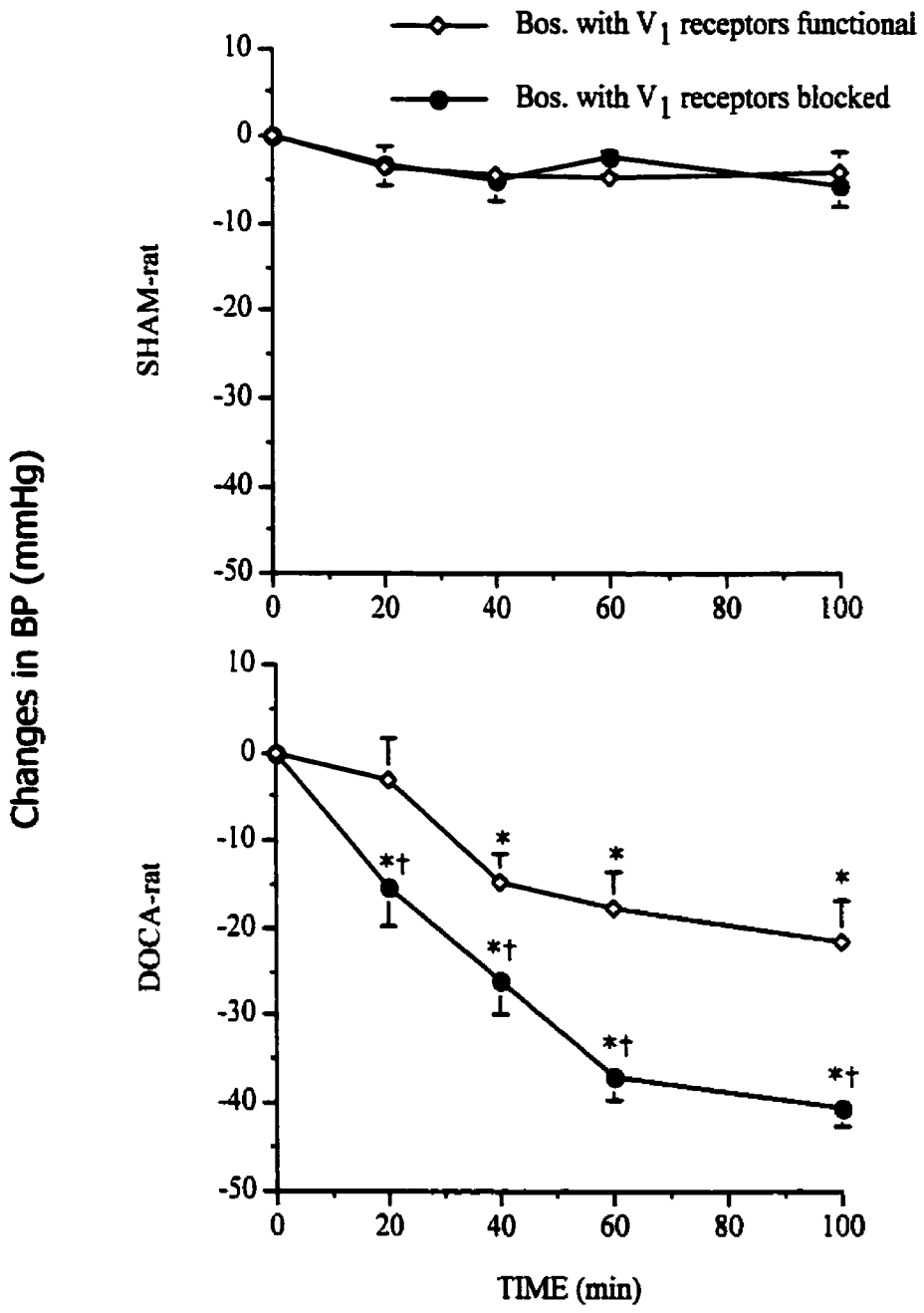

Figure 17. Changes in blood pressure (BP) in SHAM- or DOCA-salt hypertensive rats treated with bosentan with (Bos. with V_1 receptors blocked) and without the V_1 antagonist (Bos. with V_1 receptors functional) pretreatment. *P<0.05 compare to initial values; †P<0.05 compare to the effects of bosentan alone.

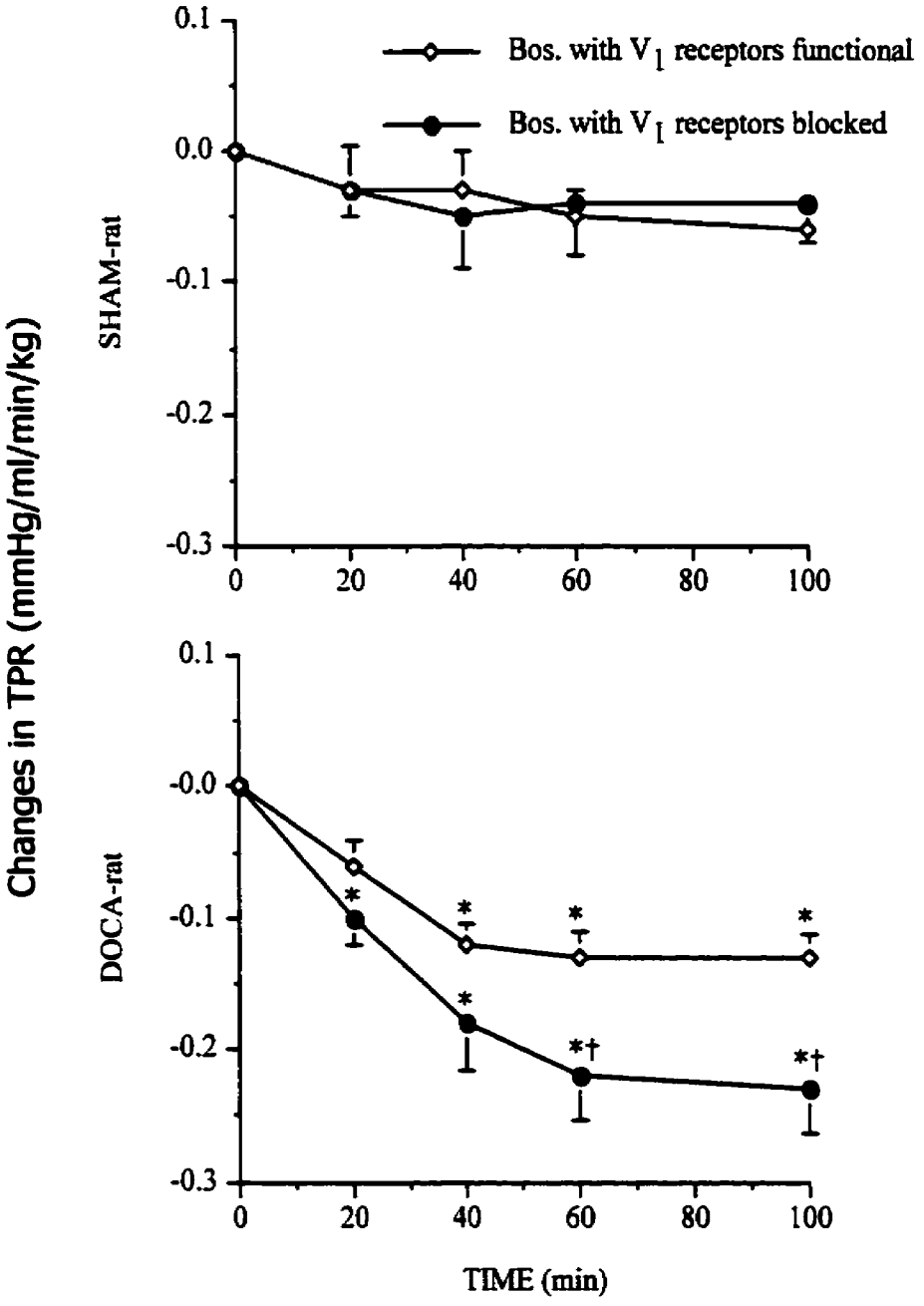

Figure 18. Changes in total peripheral resistance (TPR) in SHAM- or DOCA-salt hypertensive rats treated with bosentan with (Bos. with V_1 receptors blocked) and without the V_1 antagonist (Bos. with V_1 receptors functional) pretreatment. *P<0.05 compare to initial values; †P<0.05 compare to bosentan treatment alone.

3.3 ET dependent component of AVP in DOCA-salt hypertension

3.3.1 Control values and effects of bosentan

Control values of BP, CO and TPR in DOCA-salt hypertensive and SHAM-control rats before and after bosentan are shown in Table 6 for the AVP experiments and Table 7 for the Ang II experiments. In both series before bosentan, BP was significantly higher in DOCA-salt hypertensive rats than in SHAM-control rats. This elevated BP was due to the increased TPR, as the values for CO were not significantly different. Bosentan lowered BP in DOCA-salt hypertensive rats at 100 min after its injection. This effect was exerted at the level of resistance vessels and not on factors regulating CO, as TPR was decreased.

3.3.2 Responses to AVP

The hemodynamic responses to AVP in DOCA-salt hypertensive and SHAM-rats in the presence or absence of bosentan are shown in Fig. 19, 20 and 21. AVP induced dose-related increases in BP (Fig. 19) and TPR (Fig. 21), and decreases in CO (Fig. 20).

In the absence of bosentan, changes in TPR to all four doses of AVP were exaggerated in DOCA-rats compared to SHAM-rats (Fig. 21). Although the changes in BP evoked by AVP also tended to be higher in DOCA-salt rats than in SHAM rats, these differences were not statistically different (Fig. 19).

Treatment with bosentan blunted the increases in TPR evoked by AVP in both DOCA-salt and SHAM rats at 1, 3 and 10 ng/kg/min (Fig. 21). This blunting effect was

more dramatic in the DOCA-salt group. Consequently, the enhanced vascular responsiveness of DOCA-salt hypertensive rats to AVP observed when the ET system was functional was abolished in bosentan treated animals. Treatment with bosentan also attenuated the increases in BP evoked by AVP in both DOCA-salt and SHAM rats, at least at the 3 and 10 ng/kg/min doses. The effect of bosentan on CO was unremarkable.

Table 6: Control values before and after bosentan pretreatment in AVP study

	DOCA-rats n=32		***SHAM-rats*** n=33	
Parameter	Control	Bosentan	Control	Bosentan
BP mmHg	149±3*†	126±3	104±2	101±2
CO ml/min/kg	186±5	201±6	191±7	200±7
TPR mmHg/ml/min/kg	0.83±0.04*†	0.64±0.03	0.57±0.02	0.52±0.02

Values (means ± sem) for blood pressure (BP), cardiac output (CO) and total peripheral resistance (TPR) in SHAM-operated rats (SHAM-rats) and DOCA-salt hypertensive rats (DOCA-rats) before and 100 min after bosentan treatment. *P<0.05 compared to bosentan treatment and †P<0.05 compared to SHAM rats.

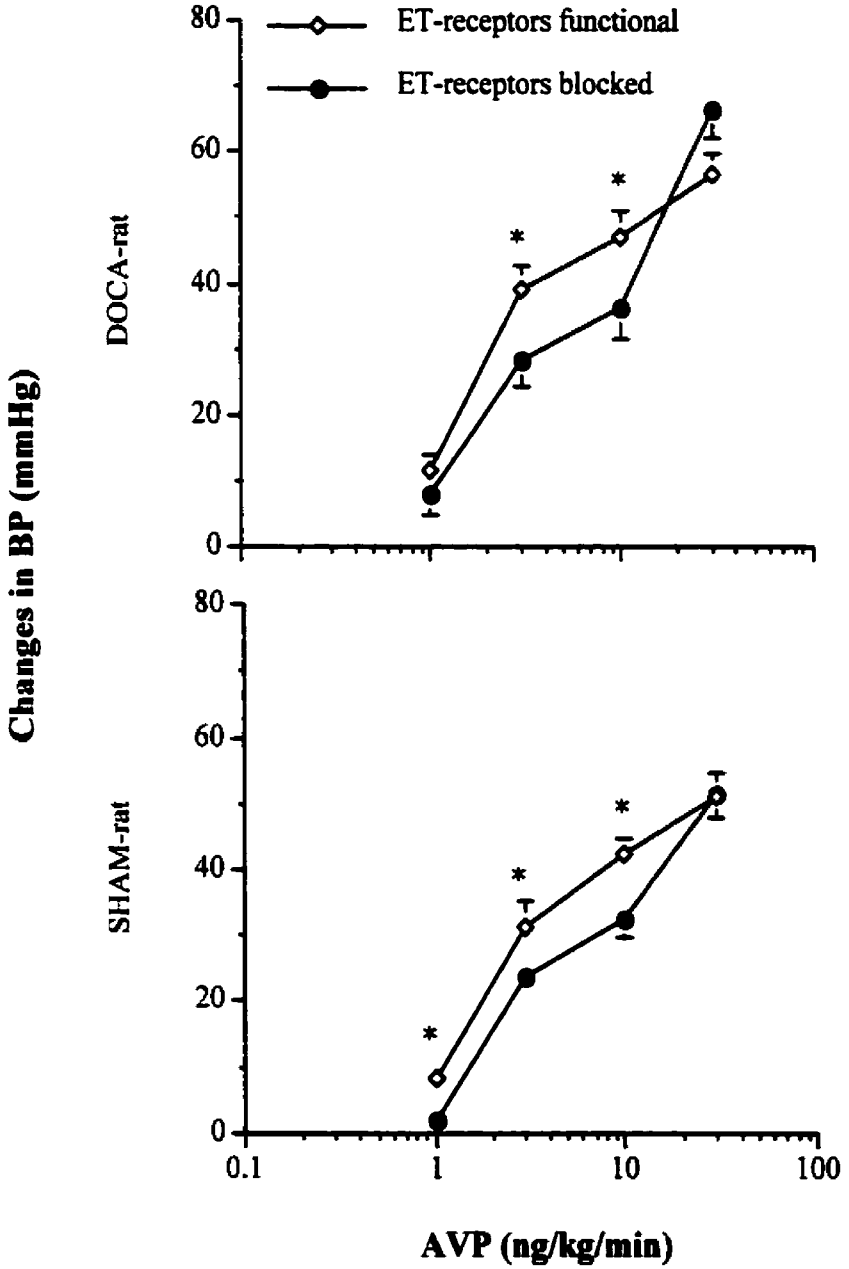

Figure 19. Changes in blood pressure to AVP in the presence (ET receptors blocked) and absence (ET receptor functional) of bosentan in DOCA-salt hypertensive and SHAM rats. *P<0.05 compared to the responses to AVP with bosentan pretreatment.

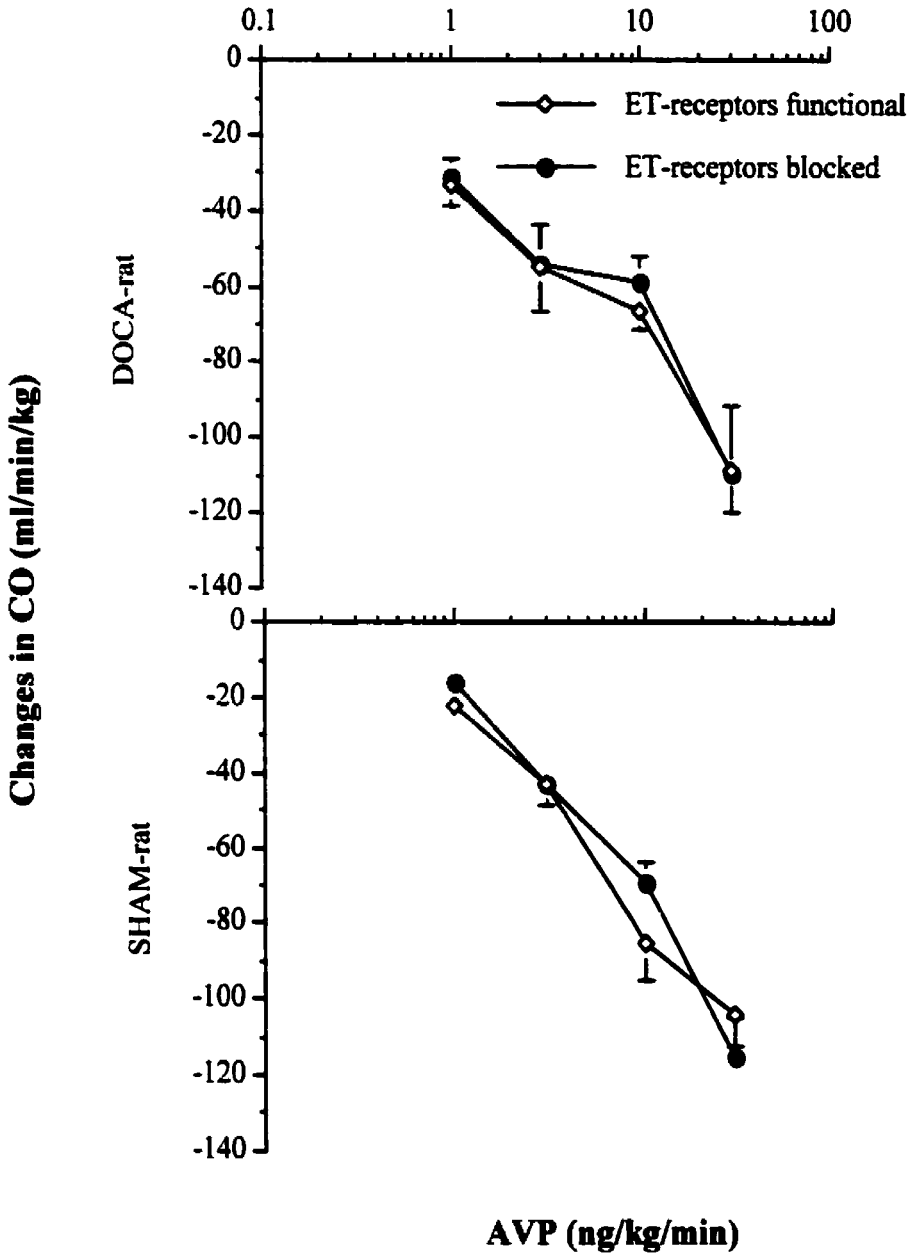

Figure 20. Changes in cardiac output to AVP in the presence (ET receptors blocked) and absence (ET receptor functional) of bosentan in DOCA-salt hypertensive and SHAM rats.

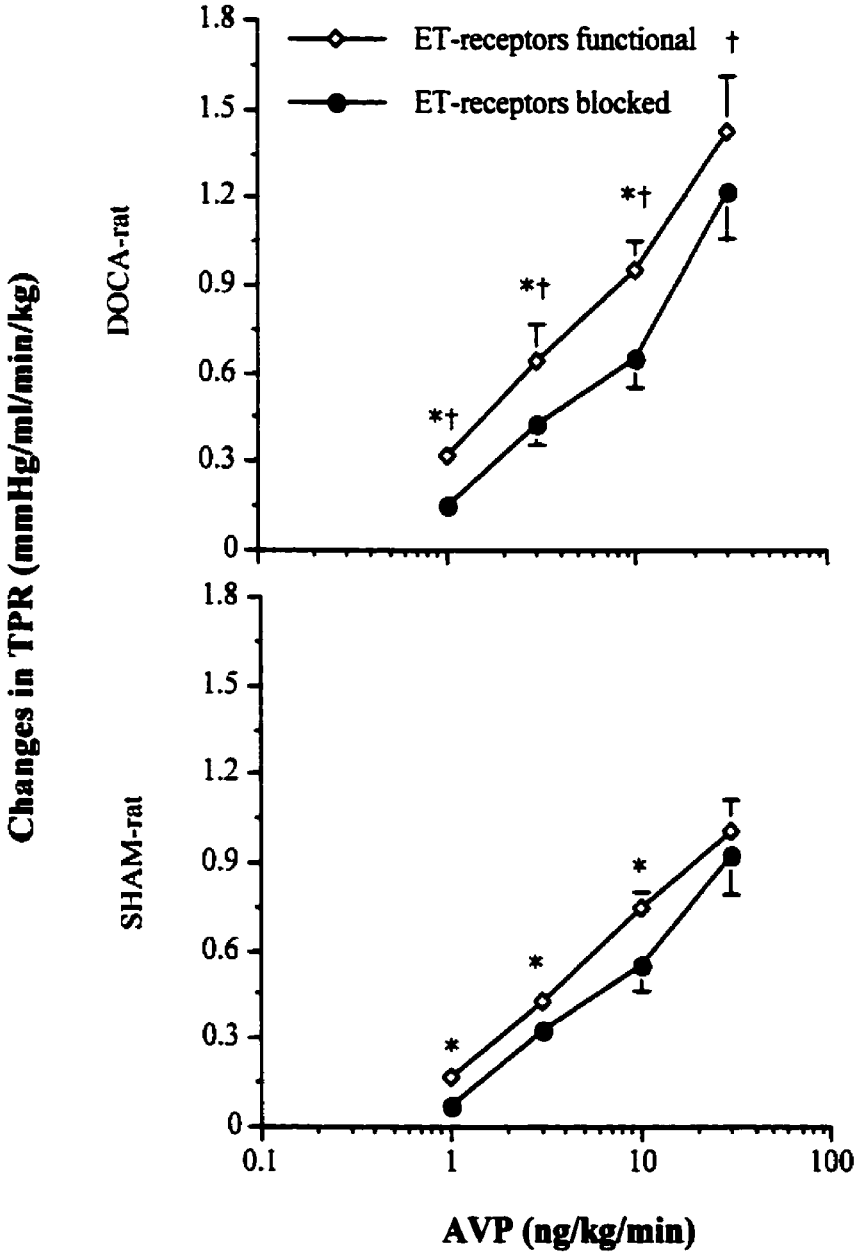

Figure 21. Changes in total peripheral resistance to AVP in the presence (ET receptors blocked) and absence (ET receptors functional) of bosentan in DOCA-salt hypertensive and SHAM rats. *P<0.05 compared to AVP responses with bosentan pretreatment and †P<0.05 compared to SHAM-rats.

3.3.3 Responses to Ang II

The hemodynamic responses to Ang II in DOCA-salt hypertensive and SHAM rats in the presence or absence of bosentan are shown in Fig. 22, 23 and 24. Ang II induced dose-related increases in BP (Fig. 22) and TPR (Fig. 24), and decreases in CO (Fig. 23).

In the absence of bosentan, changes in TPR and BP to 3, 9 and 30 ng/kg/min of Ang II were exaggerated in DOCA-rats compared to SHAM-rats (Fig. 24 and 22).

Treatment with bosentan failed to changes the responses to Ang II in both DOCA-salt and SHAM rats. Consequently, vascular and pressor responsiveness to Ang II remained elevated in the DOCA-salt hypertensive rats.

3.4 Comparison of control values

All control values in the previous three studies from DOCA-salt and SHAM-control rats, which had been treated for 3 weeks, are shown in Table 8. BP and TPR were significantly higher in DOCA-salt rats than in SHAM rats. There were no significant difference among values for each parameter in these three studies both in DOCA-salt hypertensive and SHAM-control groups.

Table 7: Control values before and after bosentan pretreatment in Ang II study

	DOCA-rats		***SHAM-rats***	
	n=25		n=26	
Parameter	Control	Bosentan	Control	Bosentan
BP mmHg	155±2*†	134±3	103±1	98±2
CO ml/min/kg	190±5	205±8	192±5	203±8
TPR mmHg/ml/min/kg	0.84±0.03*†	0.68±0.04	0.56±0.02	0.51±0.02

Values (means ± sem) for blood pressure (BP), cardiac output (CO) and total peripheral resistance (TPR) in SHAM-operated rats (SHAM-rats) and DOCA-salt hypertensive rats (DOCA-rats) before and 100 min after bosentan treatment. *P<0.05 compared to bosentan treatment and †P<0.05 compared to SHAM-control rats.

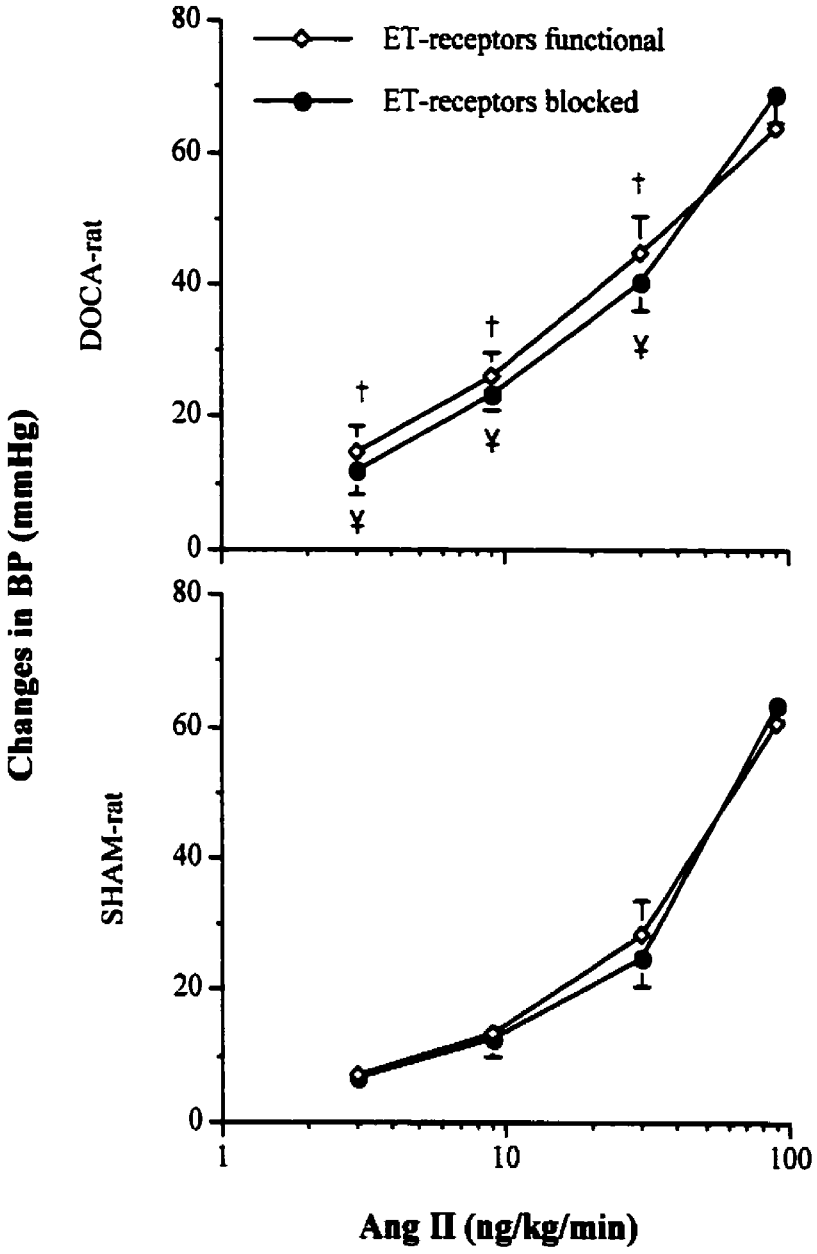

Figure 22. Changes in blood pressure to Ang II in the presence (ET receptors blocked) and absence (ET receptors functional) of bosentan in DOCA-salt hypertensive and SHAM rats. †P<0.05 compared to Ang II responses in SHAM rats in absence of bosentan and ¥<0.05 compared to SHAM rats in presence of bosentan.

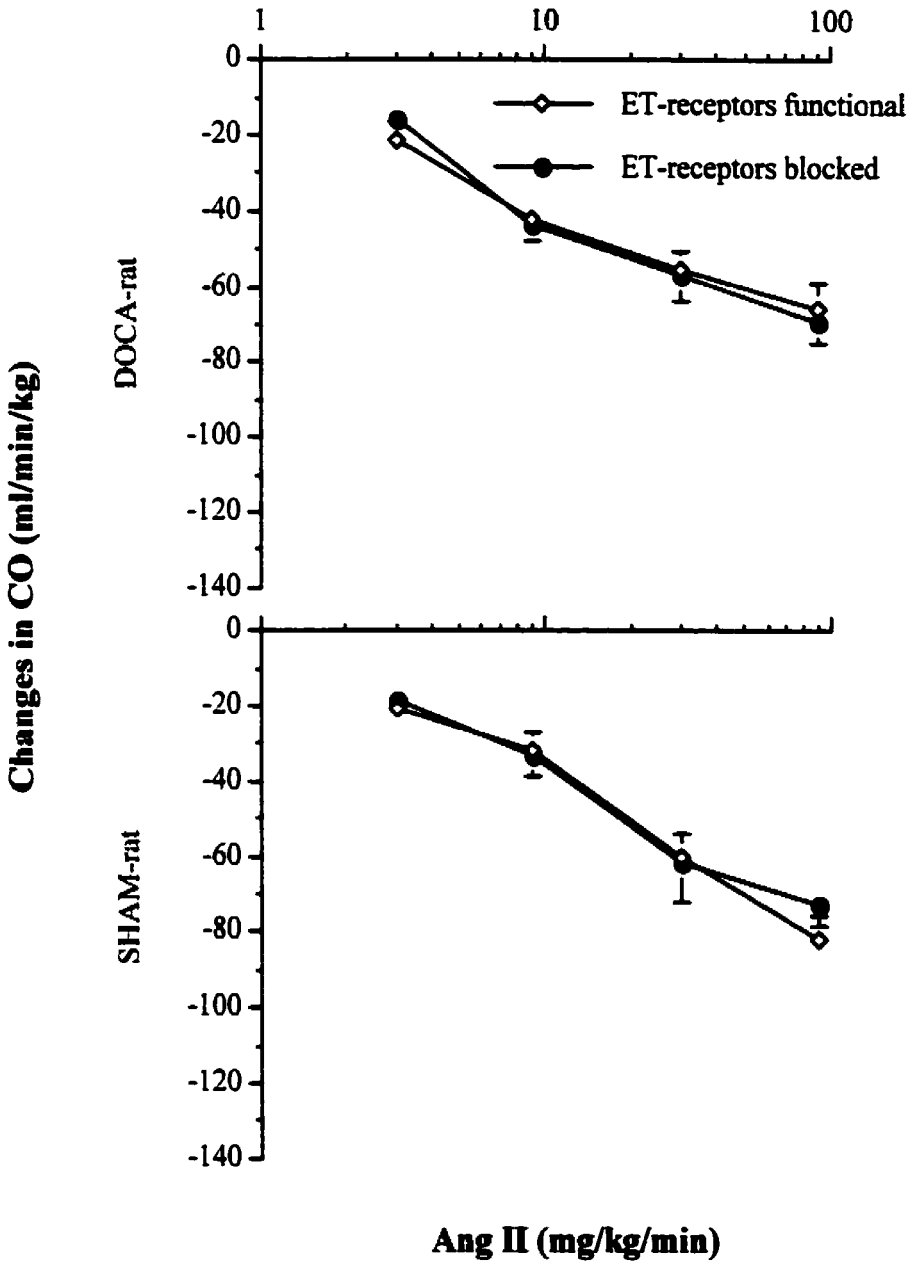

Figure 23. Changes in cardiac output to Ang II in the presence (ET receptors blocked) and absence (ET receptors functional) of bosentan in DOCA-salt hypertensive and SHAM rats.

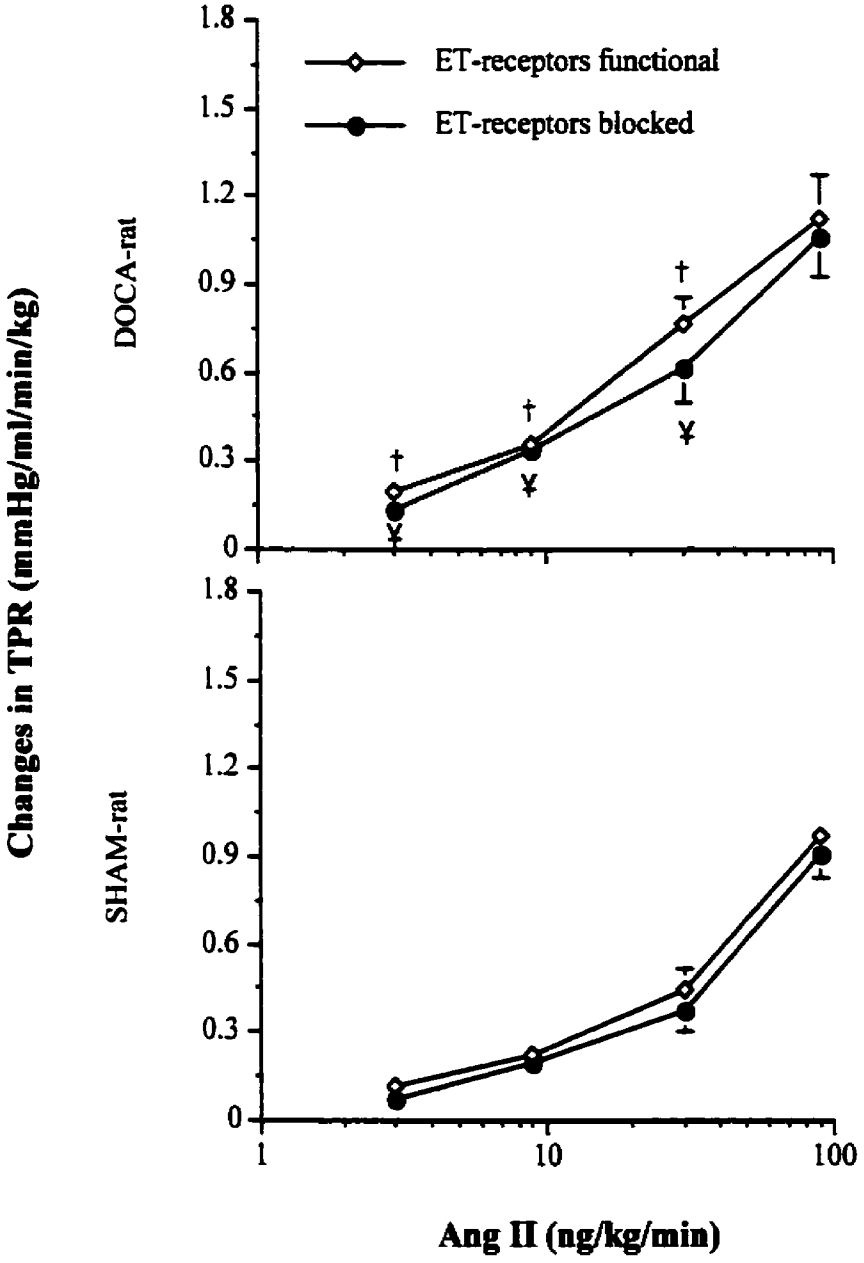

Figure 24. Changes in total peripheral resistance to Ang II in the presence (ET receptors blocked) and absence (ET receptors functional) of bosentan in DOCA-salt hypertensive and SHAM rats. †P<0.05 compared to Ang II responses in SHAM rats in absence of bosentan and ¥P<0.05 compared to SHAM rats in presence of bosentan.

Table 8: Comparison of control values: 3-week's treatment

	DOCA-rats			SHAM-rats		
	BP	CO	TPR	BP	CO	TPR
Study I	141±3*	174±8	0.84±0.04*	104±3	185±8	0.57±0.03
Study II	154±4*	186±8	0.86±0.05*	109±3	185±10	0.61±0.03
Study III	153±3*	188±5	0.84±0.04*	104±2	191±6	0.57±0.02

Control values (mean±sem) for BP (mmHg), CO (ml/min/kg) and TPR (mmHg/ml/min/kg) in DOCA-salt hypertensive and SHAM-control rats in all three studies of this thesis work. *P<0.05 compared to SHAM-rats.

4. Discussion

The experiments reported in this thesis were performed in conscious unrestrained rats. By recording BP with radiotelemetry devices and CO with chronically implanted flowprobes, hemodynamic variables could be recorded for prolonged periods without restraint and in a relatively stress-free environment. Because of problems in maintaining externalized arterial catheters patent, including problems associated with kinking and clotting, externalized catheters are typically implanted only 24 h before experiments are initiated. With telemetry implants, the instrumentation of the animals could be completed 10 days before beginning the experiments, and BP could be recorded for prolonged periods afterwards. This 10-day period served to allow a full recovery of the animals and to condition them in the recording environment.

The DOCA-salt model of hypertension was chosen for study. This model was derived from a uninephrectomized SD rat treated with DOCA and salt water, while the normotensive control rat was the uninephrectomized SD rat. The DOCA-salt hypertensive model is characterized as AVP and ET dependent, a RAS independent and volume overload form of hypertension (see section 1.2). It is the model representing human hypertension with chronic mineralocorticoid excess, such as adrenal adenoma, 11ß-hydroxylase deficiency etc. (Weber et al. 1995).

4.1 Control values

4.1.1 BP recording and its interpretation

BP recorded with radiotelemetric devices in 3-week DOCA-treated hypertensive rats in all the studies ranged from 140±3 to 155±5 mmHg. These values were lower than those reported to occur: values ranged from 157±6 to 171±5 mmHg when pressure was recorded from chronically implanted externalized catheters (Wang et al. 1993; Wang and McNeill, 1994). The differences were not due to errors in either of the methods because BP recorded by both methods simultaneously in the same animal yielded similar values at all time points in a validation study done in our lab (Balakrishnan et al. 1998). The lower BP measured by radiotelemetry in DOCA-salt hypertensive animals parallels previous works done by us and others in SHR (Balakrishnan et al. 1998; Bazil et al. 1993). The reason for lower BP in hypertensive animals recorded with radiotelemetry is unclear. It may be related to a lower level of stress and/or the longer recovery and conditioning period associated with this method (10-day's recovery with radiotelemetry vs. 1-day's recovery with external catheters). Indeed, a major advantage of telemetry is that BP can be recorded both acutely and chronically for weeks and months in conscious animals with less intervention.

The elevated BP baseline values associated with either the tail cuff method or external catheters may, in turn, affect the magnitude of responses to pharmacological interventions. Indeed, Balakrishnan et al. compared responses recorded with radiotelemetry to those recorded with externalized catheters. They found pressor

responses to infusions of AVP were diminished while antihypertensive responses accompanying withdrawal of AVP were exaggerated when BP was recorded with externalized catheters in SHR (Balakrishnan et al. 1998). Similarly, Bazil et al reported that the antihypertensive effect of captopril recorded by externalized catheters or by the tail cuff method disappeared when BP was recorded by radiotelemetry devices (Bazil et al. 1993). These results emphasize the importance of baseline BP values in quantifying responses to hypertensive or hypotensive agents and, consequently, the telemetry system in BP recording in animal research.

4.1.2 Resistance responses: importance of CO measurement

In this thesis, CO was measured with the ultrasonic transit-time system (Transonic). In contrast to electromagnetic flowmeters which have unstable zero baselines, and Doppler flowmeters which measure velocity, but not volume flow, Transonic flowmeters measure volume flow with stable zero baselines. In addition, one of most important features of the Transonic flowmeter is that it is relatively insensitive to vessel dimensions, flow profile, and flow turbulence. This flow measurement technique is a reliable and accurate method for recording flow in response to vasoactive agents in both acute and chronic experiments (Xavier et al. 1996; Wen et al. 1996).

Flow measurements are critical for evaluating vascular responses to vasoactive agents. Since BP is determined by CO and TPR, one cannot assume that changes in BP reflect changes in the resistance function of the circulation. Rather one must calculate resistance from flow and pressure recordings. Furthermore, BP is an insensitive index of vasoconstrictor activity, because an increase in TPR is often associated with a fall in CO

which attenuates the increase in BP. Indeed, AVP induces an increase in mesenteric vascular resistance and TPR at plasma concentrations within the physiological range, but this vasoconstrictor activity is not expressed as a change in BP because of a fall in CO (Montani et al. 1980; Martin and McNeill, 1987; Martin and McNeill, 1988). This is consistent with the findings reported in this thesis. The increases in TPR induced by exogenous AVP were exaggerated in DOCA-salt hypertensive rats compared to changes in TPR in SHAM rats (Fig. 21), while changes in BP were not significant difference in the two groups (Fig. 19). These results emphasize the value of flow recording in evaluating the responses of resistance vessels.

TPR was calculated as an index of the resistance function of the circulation in this thesis. In regional vascular studies *in vivo*, changes in vascular tone induced by vasoactive agents often result in changes in regional blood flow without much changes in BP, a consequence of the parallel arrangement of the various regional beds. In this case, vascular conductance, the inverse of resistance, is linearly related to flow and is a proper index to reflect changes in vascular tone unless a constant flow preparation is used (Lautt, 1989; Lautt and McQuaker, 1989). Indeed, in this situation, conductance can only decrease to zero while resistance can increase to infinity. In systemic vascular studies, vasoactive agents can induce changes in both CO and BP. If a drug evokes a proportionally greater effect on BP than on CO, then TPR, which is directly proportional to BP, is the appropriate calculation. Accordingly, TPR was selected as an index to reflect changes in systemic vascular tone in this thesis, because ET receptor antagonists induced greater changes in BP than in CO in DOCA-salt hypertensive rats, and the non

peptide mixed ET receptor antagonist, bosentan, was involved in all three studies reported in this thesis.

In all studies (Table 1- 7), BP control values were higher in DOCA-salt hypertensive rats than in SHAM rats. The elevated BP was due to a significant increase in TPR in this hypertensive model as there was no significant differences in CO between these two groups. The results are consistent with the notion that high blood pressure in established hypertension is due mainly to an increase in peripheral vascular resistance (Vanhoutte and Lüscher, 1986).

4.2 ET dependent component

4.2.1 Responsiveness to bosentan and BMS-182874

Bosentan and BMS-182874 evoked falls of arterial pressure in DOCA-salt hypertensive rats, but not in SHAM-control rats. The large decreases in BP induced by the non-selective and selective ET_A receptor antagonists indicate that the ET system, most likely through the ET_A receptor, contributes to the maintenance of hypertension in this DOCA-salt hypertensive model. The findings on the effects of a non-selective ET and a selective ET_A receptor antagonist on BP are consistent with those reported by others in the DOCA-salt model (Li et al. 1994; Doucet et al. 1996; Stein et al. 1994).

The characteristics of bosentan and BMS-182874 and their specificities are well documented (Clozel et al. 1994; Stein et al. 1994; Webb et al. 1995). Bosentan is a mixed ET antagonist which blocks both ET_A and ET_B receptors with a half-life of 5 to 8 hours in human (Ubeaud et al. 1995), while BMS-182874 is a selective ET_A receptor

antagonist. Both of them are water-soluble and are unlikely to cross the blood brain barrier. Judging from the long-lasting hypotensive effect in DOCA-salt rats compared to bosentan, BMS-182874 may have a longer half-life than bosentan. The pattern of the fall in pressure evoked by bosentan was similar to that by BMS-182874 (Fig. 1 and 4). Thus, the predominant role of endogenous ET in the DOCA-salt model is vasoconstriction, which is apparently mediated at least in part by ET_A receptors. This contrasts with the normotensive rat, in which the vasodilator effect of ET_B receptors appears to dominate (Gellai et al. 1996).

A major finding reported here is that the BP lowering effect of bosentan and BMS-182874 in the DOCA-salt hypertensive rats was exerted at the level of the resistance vessels. TPR was higher in this group (Table 1), and bosentan and BMS-182874 decreased TPR markedly in these hypertensive animals (Fig. 3 and 6). Our findings on TPR are consistent with the findings reported by others on the effects of the ET antagonist, bosentan, on regional vascular resistance (Doucet et al. 1996). In contrast to TPR, the increases in CO tended to oppose the BP lowering properties of bosentan and BMS-182774. The increase in CO was likely a consequence of the decreased afterload resulting from the vasodilator effect of ET antagonists. This study appears to be the first reporting data on the changes of TPR and CO to non-selective and selective ET_A receptor antagonists in conscious DOCA-salt hypertensive rats.

In contrast to the DOCA-salt model, bosentan and BMS-182874 had little effect on BP in normotensive rats. Some investigators have reported that the mixed ET antagonist, bosentan, or selective ET_A receptor antagonists, BQ123 and FR139317,

slightly but significantly reduced BP in normotensive rats (Doucet et al. 1996; Bigaud and Pelton, 1992; Fujita et al. 1995), while others reported that BQ123 failed to change BP (Bazil et al. 1992). These differences may be related to the experimental conditions such as the state of anesthesia (Pollock and Opgenorth, 1993), a stress-related component (Bazil et al. 1993), or genetic diversity of animals (Nabika et al. 1991). In our lab, we monitored BP in conscious unrestrained rats with radiotelemetry devices, a technique that appears to be associated with lower stress (Bazil et al. 1993), and found that bosentan and BMS-182874 fail to induce changes in BP in normotensive rats (Balakrishnan et al. 1996; Balakrishnan et al. 1997; Yu et al. 1998). These results indicate that endothelin is not essential for the control of BP in the normotensive rat. On the other hand, Gellai et al. found no evidence for a contribution of ET_A receptors to vascular tone in normotensive rats, but they did observe a significant vasodilator role for ET_B receptors (Gellai et al. 1996). Clearly, the relative contribution of ET_A and ET_B receptors appears to differ in the hypertensive and normotensive rat.

4.2.2 Vascular responsiveness to ET-1

In the DOCA-salt hypertensive rat, ET-1 content in blood vessels is elevated (Lariviere et al. 1993b) and the increases in BP and TPR to the lower doses of ET-1 were enhanced compared to SHAM-control rats (Fig. 7 and 9). These results are consistent with the notion that the vasopressor effect of ET contributes to the maintenance of vascular tone in this hypertensive animal model. Changes in vascular concentration of ET-1 result in changes in its receptors' functions. Indeed, ET receptors are

downregulated and ET-1 had been shown to evoke decreased contractile responses of aorta and mesenteric artery rings in this hypertensive animal (Nguyen et al. 1992). However, the hemodynamic response to ET-1 was enhanced in this study. In fact, the magnitude of the increases in TPR is due not only to the direct vasoconstrictor effect of ET-1 but also to the structural changes of resistance vessels observed in hypertensive animals (see section 4.4.1).

In summary, these data suggest that the ET system contributes to the maintenance of high BP and vascular tone via its vasopressor effect in DOCA-salt hypertensive rats. The maintenance of high blood pressure is exerted primarily at the level of the resistance vessels and not on factors that regulate cardiac output. Finally, the hemodynamic changes recorded with the mixed ET antagonist, bosentan, are similar to those observed with the selective ET_A antagonist, BMS-182874, suggesting that ET_A receptor function accounts for much of the blood pressure elevating properties of ET, at least in DOCA-salt hypertension.

4.3 AVP dependent component

4.3.1 Relationship of AVP and ET in maintenance of BP

Administration of the V_1 antagonist alone failed to change BP, CO and TPR (Fig. 14). There are controversial reports regarding the effect of a V_1 receptor antagonist on BP in DOCA-salt hypertensive rats (see introduction 1.2.1). This discrepancy may be attributed to experimental conditions, such as anesthesia and surgical intervention

associated with acute experiments (McNeill and Pang, 1982; Bonjour and Malvin, 1970), or stress-related components, such as BP measured by tail-cuff or externalized catheters associated with chronic experiments (Bazil et al. 1993; Balakrishnan et al. 1998). Indeed, both plasma concentration of AVP and BP were increased shortly after surgery (McNeill and Pang, 1982), and the BP-lowering effect of captopril was exaggerated in rats when BP was recorded by either the tail cuff method or the use of externalized catheters (Bazil et al. 1993). In addition, most of the previous reports were studied in DOCA-salt hypertensive rats with malignant hypertension (Crofton et al. 1979; Hiwatari et al. 1986; Filep et al. 1987), which is associated with severe complications. In this study, BP and TPR were monitored in conscious and unrestrained rats with established, but not malignant, hypertension. BP was recorded with radiotelemetry devices which allowed a long recovery period before beginning experiments (at least 10 days). Under these condition, we found that the V_1 receptor antagonist alone failed to induce any significant changes in BP and TPR in DOCA-salt hypertensive rats. The dose of the V_1 receptor antagonist used here had been shown to block responses to AVP in both DOCA-salt hypertensive rats and SHAM-control rats (Fig. 10 and 12).

The failure of the V_1 receptor antagonist alone to induce hemodynamic changes in DOCA-salt hypertensive rats (Table 4 and Fig. 14) does not exclude a role for these receptors in the maintenance of BP and TPR in this hypertensive animal model. The importance of AVP may be underestimated when only one system is the object of study. Indeed, the V_1 receptor antagonist significantly decreased BP and TPR in DOCA-salt

hypertensive rats after the blockade of the ET system (Fig. 13, 15 and 16). This hypotensive effect of the V_1 receptor antagonist was not likely due to any delayed action of bosentan because responses to bosentan had reached a plateau by this time (see section 3.1.2 and Fig. 1 and 3). These results suggest a role of AVP in the maintenance of hypertension in this experimental hypertensive rat. The observation that the V_1 receptor antagonist induced hemodynamic changes in DOCA-salt hypertension only after the blockade of ET system suggests that the ET system compensates to maintain blood pressure when the V_1 receptors were blocked.

Conversely, it appears the AVP system compensates in responses to blockade of the ET system in order to support blood pressure because the hemodynamic changes to bosentan were exaggerated in this hypertensive animal in the presence of the V_1 antagonist (Fig. 14, 17 and 18). Together, the data obtained from the two series of experiments in which the AVP system and ET system were blocked sequentially reveal the reciprocal or redundant nature of the ET and AVP systems in maintenance of systemic hemodynamics in DOCA-salt hypertensive rats. In the absence of one system, the other compensates fully, and it is only when both systems were the object of study that the importance of each became evident. Thus, the failure of an antagonist to reduce BP must be interpreted cautiously.

Interestingly, blockade either of AVP or ET receptors alone did not induce any significant changes in BP and TPR in SHAM-control rats (Table 3 and 4, Fig. 13 and 14). This observation suggests that ET and AVP are not essential in maintenance of BP in these normotensive rats. However, when these normotensive rats were treated with

both bosentan and the V_1 receptor antagonist concurrently, BP and TPR decreased significantly, although the changes were small (Fig. 13 and 14). These results further demonstrate a reciprocal relationship between the ET system and AVP systems.

In summary, the synergetic effects of bosentan and the V_1 receptor antagonist suggest that the ET and AVP systems are overlapping mechanisms that play a reciprocal role in the maintenance of systemic hemodynamics in conscious DOCA-salt hypertensive rats: when one system is inhibited, the other compensates to maintain it.

4.3.2 Effectiveness of bosentan and [d(CH$_2$)$_5$^{1}, O-Me-Tyr^2,Arg^8]-vasopressin

The doses of bosentan and [d(CH$_2$)$_5$^{1}, O-Me-Tyr^2,Arg^8]-vasopressin were chosen from the literature. Nevertheless, in order to prove that the compounds in our possession were active, we tested their ability to block the responses evoked by ET-1 and AVP. The dose-related increases in BP and TPR evoked by ET-1 in both DOCA-salt and SHAM rats were markedly attenuated by bosentan (Fig. 7 and 9). The dose-related increases in BP and TPR evoked by AVP in both DOCA-salt and SHAM rats were virtually abolished by [d(CH$_2$)$_5$^{1}, O-Me-Tyr^2,Arg^8]-vasopressin (Fig. 10 and 12).

4.3.3 Baseline effect of bosentan to ET-1

It could be argued that the effects of bosentan on responsiveness to ET-1 were influenced by different baseline values. Bosentan caused a marked reduction in TPR and BP in DOCA-salt hypertensive rats, and the dose-response relationships to ET-1 in the presence of bosentan were recorded from this lower baseline compared to the response in the absence of bosentan. However, this effect of bosentan alone should, if anything,

exaggerate responses to ET-1 since a lower baseline leaves a greater potential for a vasoconstrictor or pressor response. Indeed, there is data to show that a lower baseline tends to exaggerate pressor responses (Balakrishnan et al. 1998) and to diminish depressor responses (Bazil et al. 1993; Balakrishnan et al. 1998). Thus, if anything, the effectiveness of the blockade with bosentan would be underestimated by the baseline effect.

4.4 Contribution of ET to AVP evoked responses

4.4.1 Vascular responsiveness to AVP and Ang II

Vascular responsiveness to AVP appeared to be enhanced in DOCA-salt rats at least as judged by the changes in TPR: the increases in TPR evoked by AVP were greater in DOCA-salt hypertensive rats than in SHAM control rats (Fig. 21). This finding on vascular reactivity, together with the fact that plasma AVP is increased in this hypertensive rat model (Mohring et al. 1977; Crofton et al. 1979), provide additional evidence implicating AVP in maintenance of vascular tone in DOCA-salt hypertensive rats.

In contrast to the data shown in Fig. 21, the data shown in Fig. 12 suggests that except for the lowest dose of AVP, vascular responsiveness to AVP was not enhanced in the DOCA-salt group compared to SHAM rats. However, the data in Fig. 12 were derived from a cumulative dosage design in 2-week DOCA-salt animals compared to the single dosage design in 3-week DOCA-salt rats for the data reported in Fig. 21. These

differences might be related to experimental protocols (a single-dose infusion vs. infusions in a cumulative fashion) and/or the severity of hypertension (3-week vs. 2-week's DOCA and salt treatment). Indeed, Matsuguchi and Schmid did find that the BP responses to AVP were related to the duration of the hypertensive regimen in the DOCA-salt animal (Matsuguchi and Schmid, 1982). In the final analysis, the data on single infusions in the 3-week's DOCA-salt treated hypertensive rats would be seem to be the more relevant.

While changes in TPR to AVP were enhanced in this hypertensive rat, changes in BP were not enhanced. This result further emphasizes the importance of TPR monitoring to evaluate hemodynamic responses to a vasoactive agent. The reason that the changes in vascular reactivity were not translated into changes in pressor activity are unknown, but may be related to a specific enhancement in baroreflex activity associated with elevations in the circulating levels of AVP in the DOCA-salt hypertensive animal (see section 1.1.1.1.2).

Vascular responsiveness to Ang II was also enhanced in DOCA-salt hypertensive rats: the changes in TPR evoked by Ang II were greater in DOCA-salt hypertensive rats than in SHAM control rats. In DOCA-salt hypertensive rats, plasma renin activity is markedly lower and vascular Ang II receptors are up-regulated (Schiffrin et al. 1983). This increased receptor density could contribute to the enhanced vascular responsiveness. In contrast to AVP, the enhanced vascular responsiveness was expressed as an increase in pressor responsiveness. This finding is perhaps not surprising since Ang

II does not enhance baroreflex activity. Indeed, it may attenuate reflexes (Guo and Abboud, 1984).

Similar to AVP and Ang II, vascular responsiveness to ET-1 was also enhanced in DOCA-salt hypertensive rats. This enhanced responsiveness was associated with enhanced pressor responsiveness, at least at the two lower doses of ET-1 (Fig. 7 and 9).

The observation that vascular responsiveness was enhanced to all these peptides, AVP, ET-1 and Ang II, suggested that the increase in vascular reactivity is non-specific. Responsiveness to vasoactive agents depends not only on its interaction with receptors, but also on the vascular structure of resistance vessels. Folkow et al (Folkow et al. 1973; Folkow, 1982) proposed that the augmented vascular reactivity in hypertension is due a decrease in the lumen to wall thickness ratio of resistance vessels. Thickening of the arteriolar wall occurs due to hypertrophy or hyperplasia of vascular smooth muscle cells in the media of blood vessels. Indeed, vascular hypertrophy is very severe in resistance arteries from DOCA-salt hypertensive rats, with a prominent thickening of the media (Deng and Schiffrin, 1992). The remodeled resistance arteries exhibit a reduced circumference, which acts as an amplifier to vasoconstrictor activity. This structurally based amplification may explain the difference between the enhanced TPR responses observed *in vivo* in this study and the attenuated contractile effect to ET-1 reported to occur in blood vessels rings preparation (Nguyen et al. 1992). These attenuated contractile responses to ET-1 *in vitro* might be amplified by the vessel remodeling, resulting in normal or augmented pressor responses *in vivo*.

In contrast to Ang II and ET-1, the enhanced vascular responses to AVP were not expressed as an increased pressor responsiveness. In fact, pressor responsiveness to AVP is complex and it is buffered by increased baroreflexes and decreased CO which might offset any non specific structurally-induced enhancement of vascular responsiveness (see introduction 1.1.1.1)

4.4.2 Contribution of ET to the hemodynamic effects of AVP

The blunted responsiveness of both DOCA-salt hypertensive and SHAM control rats to AVP in the presence of bosentan (Fig. 19 and 21) is the major finding in this part of the thesis (Result 3.3). The data here provide direct evidence at the hemodynamic level that ET contributes to the pressor activity of AVP in this hypertensive animal model and its normotensive control. For reasons discussed earlier (see section 4.3.3), the magnitude of the contribution of ET was likely underestimated in the hypertensive group because bosentan lowered BP in this group (126 vs. 149 mmHg in Table 6) providing a greater potential for a BP elevating response to AVP in the bosentan treated hypertensive animals.

The observation also demonstrates that this contribution of ET to the pressor activity of AVP is exerted at the level of resistance vessels, as TPR responses to AVP were decreased when the ET system was blocked (Fig. 21). In contrast, decreases in CO tended to oppose the BP elevating effect resulting from the vasoconstrictor activity of the peptide, and bosentan failed to change CO responses to AVP (Fig. 20).

The data is consistent with work demonstrating release of ET-1 by AVP. AVP has been shown to increase ET-1 release in cultured endothelial cells, from perfused

mesenteric arteries, and in a whole animal study (Emori et al. 1991; Tomobe et al. 1993a; Emmeluth and Bie, 1992). In addition to release of ET-1, ET-1 gene expression and ET-1 content of blood vessels are elevated in DOCA-salt hypertensive rats (Lariviere et al. 1993b; Lariviere et al. 1993a), and chronic treatment with OPC-21268, a V_1 receptor antagonist, attenuated the enhanced ET-1 gene expression in this hypertensive model (Intengan et al. 1998). Finally, plasma levels of AVP are elevated in the DOCA-salt animal model (Mohring et al. 1977; Yamamoto et al. 1984; Filep et al. 1987). Accordingly, it is reasonable to postulate that AVP contributes to the hypertensive state indirectly by increasing ET-1 gene expression and by releasing ET-1.

Similar to DOCA-salt hypertensive rats, ET also contributed to the changes in TPR evoked by AVP in SHAM control SD rats. The contribution was more profound in DOCA-salt hypertensive rats than in SHAM rats (Fig. 21). By contrast, ET did not contribute to the hemodynamic responses to AVP in a different normotensive strain, namely WKY rats (Balakrishnan et al. 1997). This discrepancy in normotensive rats might be caused by the strain difference, WKY vs. SD rats. Indeed, the pressor response to AVP was significantly higher in SD rats than in WKY rats (Tatchum-Talom and McNeill, 1997).

The conclusions of this study depend on the assumption that bosentan is a selective ET antagonist, and also that the dose employed would antagonize the ET system in DOCA-salt hypertensive rats and SHAM control rats to a similar extent. The pharmacological characteristics of bosentan have been described in detail by Clozel et al (Clozel et al. 1994): the compound competitively inhibited the specific binding of ET-1

to a variety of cell types, but failed to affect the binding of 40 other peptides including AVP. Moreover, the dose of bosentan used in this study has been tested to attenuate the effects of ET-1 in both rat groups (Fig. 7 and 9).

In contrast to AVP, bosentan failed to blunt the increases in BP and TPR elicited by Ang II both in DOCA-salt hypertensive rats and SHAM control rats (Fig. 22 and 24), suggesting that the contribution of ET to the hemodynamic responses to AVP may be specific in this hypertensive model. There are controversial reports regarding the effect of Ang II on ET-1 secretion from cultured cells (Emori et al. 1991; Hieda and Gomez-Sanchez, 1990; Essig et al. 1997). The reason for this is unknown. It might be due to the origin of cells, i.e. from different tissues or from different species. *In vivo*, Emmeluth and Bie reported that infusion of AVP, but not Ang II, increased plasma ET-1 levels in conscious dogs (Emmeluth and Bie, 1992). However, Balakrishnan et al. from our lab showed that bosentan significantly blunted pressor responses to lower doses of Ang II both in SHR and WKY rats (Balakrishnan et al. 1996), indicating the involvement of ET in the responses to Ang II. The differences in the involvement of ET in the hemodynamic responses to Ang II between DOCA-salt hypertensive rats and SHR may be related to plasma renin activity, humoral factors, and/or genetic diversity.

In summary, the results suggest that ET contributes to the hemodynamic effects of AVP, but not Ang II, in both DOCA-salt hypertensive rats and SHAM-controlled SD rats. This contribution is exerted at level of the resistance function of the circulation, and not on factors that regulate CO.

4.5 Conclusion and Summary

The roles of the ET system and AVP system, and the interaction between these systems in maintenance of systemic hemodynamics of DOCA-salt hypertensive rats and SHAM control rats were examined in experiments described in this thesis. BP was recorded with the radiotelemetry, a technique which is associated with lower level of stress and which is suitable both for acute and chronic experiments. CO was monitored with the Transonic system, which measures volume flow, instead of flow velocity. Using these techniques, we were able to record BP and CO in conscious unrestrained rats 10 days after recovery from surgery and for 2 weeks afterwards.

In contrast to SHAM-controlled rats, bosentan, a mixed ET antagonist, and BMS-182874, a selective ET_A antagonist, lowered BP dramatically in the DOCA-salt hypertensive animal. This hypotensive effect of ET antagonists was due to a decrease in TPR, not to factors regulating CO, since CO was increased by ET antagonists. The magnitude and time-course profile of the hypotensive properties of bosentan and BMS-182874 were similar. These findings suggest that the contribution of ET can be accounted for by its effects on ET_A receptors located on resistance vessels of the circulation.

Administration of the V_1 receptor antagonist alone failed to induce significant changes in DOCA-salt hypertensive rats and SHAM-controlled rats. However, the V_1 receptor antagonist decreased BP and TPR significantly when the ET system was blocked and prevented from compensating in this hypertensive animal. Conversely, the responses to bosentan were significantly exaggerated after the blockade of V_1 receptors

compared to the responses to bosentan alone in the DOCA-salt hypertensive animal. These results demonstrate the involvement of AVP in maintenance of systemic hemodynamics via its vasoconstrictor effects mediated by V_1 receptors, and also reveal the reciprocal or redundant relationship between the AVP system and ET system in the control of systemic hemodynamics in the DOCA-salt hypertension.

In contrast to Ang II, the vasopressor responses to AVP were blunted by bosentan both in DOCA-salt hypertensive rats and SHAM-controlled rats, but more profoundly in the former. This provides direct evidence at hemodynamic level for an ET component contributing to the pressor responsiveness to AVP. The contribution of ET to pressor activity of AVP was exerted at the level of the resistance vessels, not on factors which regulate CO.

In conclusion, the results of this thesis demonstrate the involvement of both the ET system and AVP system, and the reciprocal or redundant nature between these two systems in the control of systemic hemodynamics in DOCA-salt hypertensive rats. The results also demonstrate a contribution of ET to pressor activity of AVP in DOCA-salt hypertension.

References

AGRE, P., PRESTON, G. M., SMITH, B. L., JUNG, J. S., RAINA, S., MOON, C., GUGGINO, W. B., and NIELSEN, S. 1993. Aquaporin CHIP: the archetypal molecular water channel. Am. J. Physiol. **265**: F463-F476.

AGRE, P., BROWN, D., and NIELSEN, S. 1995. Aquaporin water channels: unanswered questions and unresolved controversies. Curr. Op. Cell Biol. **7**: 472-483.

AGRICOLA, K., RUBANYI, G., PAUL, R. J., and HIGHSMITH, R. F. 1984. Characterization of a potent coronary artery vasoconstrictor produced by endothelial cells in culture. Fed. Proc. **43**: 899.(Abstract)

AKI, Y., TAMAKI, T., KIYOMOTO, H., HE, H., YOSHIDA, H., IWAO, H., and ABE, Y. 1994. Nitric oxide may participate in V_2 vasopressin-receptor-mediated renal vasodilation. J. Cardiovasc. Pharmacol. **23**: 331-336.

ALESCIO-LAUTIER, B., METZGER, D., and SOUMIREU-MOURAT, B. 1993. Central behavioral effects of vasopressin: point and perspective. Rev. Neurosci. **4**: 239-266.

ALTURA, B. M. 1973. Selective microvascular constrictor actions of some neurohypophyseal peptides. Eur. J. Pharmacol. **24**: 49-60.

ALTURA, B. M. 1975. Dose-response relationships for arginine vasopressin and synthetic analogs on three types of rat blood vessels : possible evidence for regional differences in vasopressin receptor sites within a mammal. J. Pharmacol. Exp. Ther. **193**: 413-423.

ALTURA, B. M. and ALTURA, B. T. 1977. Vascular smooth muscle and neurohypophyseal hormones. Federation Proceedings **36**: 1853-1860.

ALTURA, B. M. 1978. Pharmacology of venular smooth muscle: new insights. Microvasc. Res **16(1)**: 91-117.

ALTURA, B. M. and ALTURA, B. T. 1984. Actions of vasopressin, oxytocin, and synthetic analogs on vascular smooth muscle. Fed. Proc. **43 (1)**: 80-86.

ANDREATTA-VAN-LEYEN, S., AVERILL, D. B., and FERRARIO, C. M. 1990. Cardiovascular actions of vasopressin at the ventrolateral medulla. Hypertension **15 (2 Suppl)**: I-102-I-106.

AONO, J., KOGA, T., and YAMAZAKI, T. 1988. Antihypertensive action of a novel orally active angiotensin converting enzyme inhibitor altiopril calcium (MC838) in several hypertensiv models of rats: comparison with captopril. Arch. Int. Pharmacodyn. Ther. **292**: 203-222.

ARAI, H., HORI, S., ARAMORI, I., OHKUBO, H., and NAKANISHI, S. 1990. Cloning and expression of a cDNA encoding a non-isopeptide-selective sub-type of the endothelin receptor . Nature **348**: 732-735.

ARMSTEAD, W. M. 1996. Influence of brain injury on vasopressin-induced pial artery vasodilation: role of superoxide anion. Am. J. Physiol. **270**: H1272-H1278.

AYLWARD, P. E., FLORAS, J. S., LEIBACH, W. N., and ABBOUD, M. D. 1986. Effects of vasopressin on the circulation and its baroreflex control in healthy men. Circulation **73**: 1145-1154.

BADR, K. F., MURRAY, J. J., BREYER, M. D., TAKAHASHI, K., INAGAMI, T., and HARRIS, R. C. 1989. Mesangial cell, glomerular and renal vascular responses to endothelin in the rat kidney. Elucidation of singnal trasduction pathways. J. Clin. Invest. **83**: 336-342.

BAKRIS, G. L., FAIRBANKS, R., TRAISH, A. M., AKERSTROM, V., and KERN, S. 1991. Arginine vasopressin stimulates human mesangial cell production of endothelin. J. Clin. Invest. **87**: 1158-1164.

BAKRIS, G. L. and RE, R. N. 1993. Endothelin modulates angiotensin-II induced mitogenesis of human mesangial cells. Am. J. Physiol. **264**: F937-F942.

BALAKRISHNAN, S. and MCNEILL, J. R. 1996. Cardiac output mediates the antihypertensive effect of vasopressin in spontaneous hypertension. Am. J. Physiol. **271**: H1728-H1733.

BALAKRISHNAN, S., WANG, H. D., GOPALAKRISHNAN, V., WILSON, T. W., and MCNEILL, J. R. 1996. Effect of an endothelin antagonist on hemodynamic responses to angiotensin II. Hypertension **28**: 806-809.

BALAKRISHNAN, S., GOPALAKRISHNAN, V., and MCNEILL, J. R. 1997. Endothelin contributes to the hemodynamic effects of vasopressin in spontaneous hypertension. Eur. J. Pharmacol. **334**: 55-60.

BALAKRISHNAN, S., TATCHUM-TALOM, R., and MCNEILL, J. R. 1998. Radiotelemetric versus externalized catheter monitoring of blood pressure: Effect of vasopressin in spontaneous hypertension. J. Pharmacol. Toxicol. Methods **40**: 87-93.

BAZIL, M. K., LAPPE, R. W., and WEBB, R. L. 1992. Pharmacologic characterization of an Endothelin$_A$ (ET_A) receptor antagonist in conscious rats. J. Cardiovasc. Pharmacol. **20**: 940-948.

BAZIL, M. K., KRULAN, C., and WEBB, R. L. 1993. Telemetric monitoring of cardiovascular parameters in conscious spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. **22**: 897-905.

BERECEK, K. H., MAH, S. C., BAUM, P., and HOFBAUER, K. G. 1984a. Cardiovascular effect of intracerebroventricular injection of vasopressin in unanaesthetized normotensive and DOCA-salt hypertensive rats. Am. J. Hypertens. **2 (suppl 3)**: 29-31.

BERECEK, K. H., OLPE, H. R., JONES, R. S. G., and HOFBAUER, K. G. 1984b. Microinjection of vasopressin into the locus coeruleus rats. Am. J. Physiol. **247 (4 pt 2)**: H675-H681.

BIGAUD, M. and PELTON, J. T. 1992. Discrimination between ET_A- and ET_B-receptor-mediated effects of endothelin-1 and [$Ala^{1,3,11,15}$]endothelin-1 by BQ-123 in the anaesthetized rat. Br. J. Pharmac. **107**: 912-918.

BIRD, J. E., MORELAND, S., WALDRON, T. L., and POWELL, J. R. 1995. Antihypertensive effects of a novel endothelin-A receptor antagonist in rats. Hypertension **25**: 1191-1195.

BONJOUR, J. P. and MALVIN, R. L. 1970. Plasma concentrations of ADH in conscious and anesthetized dogs. Am. J. Physiol. **218**: 1128-1132.

BOUVIER, M. and CHAMPLAIN, J. DE 1986. Increased Sympatho-Adrenal Tone and Adrenal Medulla Reactivity in DOCA-Salt Hypertensive Rats. J. Hypertens. **4**: 157-163.

BOYLE, W. A. and SEGAL, L. D. 1986. Direct cardiac effects of vasopressin and their reversal by a vascular antagonist. Am. J. Physiol. **251**: H734-H741.

BRATTSTROM, A., DE JONG, W., and DE WIED, D. 1988. Vasopressin micro-injection into the nucleus tractus solitarii decrease heart rate and blood pressure in anesthetized rats. J. Hypertens. **6 (4)**: S521-S524.

BRIMBLE, M. J., FORSLING, M. L., and MUSABAYANE, C. T. 1988. Natriuretic action of vasopressin in the conscious unrestrained rat. Acta Endocrinologica **119**: 386-390.

BUDZIKOWSKI, A. S., PACZWA, P., and SZCZEPANSKA-SADOWSKA, E. 1996. Central V_1 AVP receptors are involved in cardiovascular adaptation to hypovolemia in WKY but not in SHR. Am. J. Physiol. **271**: H1057-H1064.

BURNIER, H., BIOLLAZ, J., BRUNNER, D. B., GAVRAS, H., and BRUNNER, H. R. 1983. Alpha and beta adrenoceptor blockade in normotensive and deoxycorticosterone (DOC)-hypertensive rats; plasma vasopressin and vasopressin pressor effect. J. Pharmacol. Exp. Ther. **224**: 222-227.

BURRELL, L. M., PHILLIPS, P. A., STEPHENSON, J. M., RISVANIS, J., ROLLS, K. A., and JOHNSTON, C. I. 1994. Blood pressure-lowering effect of an orally active vasopressin V1 receptor antagonist in mineralocorticoid hypertension in the rat. Hypertension **23**: 737-743.

CABRAL, A. M., CARVALHINHO, F. B., and VASQUEZ, E. C. 1994. Effects of chlorthalidone on ventricular hypertrophy in deoxycorticosterone acetate salt hypertensive rats. Hypertension **23**: 1180-1184.

CHAN, W. Y., WO, N. C., STOEV, S., CHENG, L. L., and MANNING, M. 1998. Discovery of novel selective hypotensive vasopressin peptides that exhibit little or no functional interactions with known oxytocin/vasopressin receptors. Br. J. Pharmacol. **125**: 803-811.

CHAROCOPOS, F., HATZINIKOLAOU, P., NORTH, W. G., and GAVRAS, H. 1982. Systemic and regional hemodynamic effects of endogenous vasopressin stimulation in rats. Am. J. Physiol. **243 (4)**: H560-H565.

CHEN, L., MCNEILL, J. R., WILSON, T. W., and GOPALAKRISHNAN, V. 1995a. Differential effects of phosphoramidon on contractile responses to angiotensin II in rat blood vessels. Br. J. Pharmacol. **114**: 1599-1604.

CHEN, L., MCNEILL, J. R., WILSON, T. W., and GOPALAKRISHNAN, V. 1995b. Heterogeneity in vascular smooth muscle responsiveness to angiotensin II: Role of endothelin. Hypertension **26**: 83-88.

CHENG, C. -P., IGARASHI, Y., KLOPFENSTEIN, H. S., APPLEGATE, R. J., SHIHABI, Z., and LITTLE, W. C. 1993. Effect of vasopressin on left ventricular performance. Am. J. Physiol. **264**: H53-H60.

CHIU, E. K. Y. and MCNEILL, J. R. 1985. Vasopressin withdrawal produces hypotension in the spontaneously hypertensive rat. Am. J. Physiol. **249**: H193-H197.

CHIU, E. K. Y. and MCNEILL, J. R. 1986. The effect of prolonged infusion and withdrawal of angiotensin II in the spontaneously hypertensive rat. Can. J. Physiol. Pharmacol. **64**: 748-750.

CHIU, E. K. Y. and MCNEILL, J. R. 1987. Pretreatment with vasodilator or V1-antagonist abolishes vasopressin withdrawal hypotension in spontaneously hypertensive rats. J. Hypertens. **5**: 593-598.

CHIU, E. K. Y. and MCNEILL, J. R. 1989a. Withdrawal-induced antihypertensive effect of vasopressin in doca-salt hypertension. Am. J. Hypertens. **2**: 458-461.

CHIU, E. K. Y. and MCNEILL, J. R. 1989b. Antihypertensive effect of vasopressin withdrawal in young and adult spontaneously hypertensive rats. Hypertension **14**: 66-72.

CHIU, E. K. Y. and MCNEILL, J. R. 1995. Sympathectomy exaggerates the antihypertensive effect of vasopressin withdrawal. Am. J. Physiol. **268**: H1-H6.

CLOZEL, M. and CLOZEL, J. P. 1989. Effects of endothelin on regional blood flows in squirrel monkeys. J. Pharmacol. Exp. Ther. **250**: 1125-1131.

CLOZEL, M., BREU, V., GRAY, G. A., KALINA, B., LÖFFLER, B. -M., BURRI, K., CASSAL, J. -M., HIRTH, G., MÜLLER, M., NEIDHART, W., and RAMUZ, H. 1994. Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. J. Pharmacol. Exp. Ther. **270**: 228-235.

COWLEY, A. W.,JR., MONOS, E., and GUYTON, A. C. 1974. Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. Circ. Res. **39**: 779-787.

COWLEY, A. W.,JR. and BARBER, B. 1983. Vasopressin vascular and reflex effects-a theoretical analysis. Progress in Brain Research **60**: 415-424.

COWLEY, A. W.,JR., MERRILL, D., OSBORN, J., and BARBER, B. J. 1984a. Influence of vasopressin and angiotensin on baroreflexes in the dog. Circ. Res. **54**: 163-172.

COWLEY, A. W.,JR., MERRILL, D. C., QUILLEN, E. W., and SKELTON, M. M. 1984b. Long-term blood pressure and metabolic effects of vasopressin with servo-controlled fluid volume. Am. J. Physiol. R537-R545.

COWLEY, A. W.,JR., SZCZEPANSKA-SADOWSKA, E., STEPNIAKOWSKI, K., and MATTSON, D. 1994. Chronic intravenous administration of V_1 arginine vasopressin agonist results in sustained hypertension. Am. J. Physiol. Heart Circ. Physiol. **267**: H751-H756.

COWLEY, A. W.,JR. and ROMAN, R. J. 1996. The Role of the Kidney in Hypertension. JAMA **275**: 22-29.

COWLEY, A. W.,JR., SKELTON, M. M., and KURTH, T. M. 1998. Effects of long-term vasopressin receptor stimulation on medullary blood flow and arterial pressure. Am. J. Physiol. **275**: R1420-R1424.

Cowley, A.W.,Jr. and Liard, J-F. (1987) *Cardiovascular actions of vasopressin; in Vasopressin, Principles and Properties*, 389-433, New York and London, Plenum press.

COX, B. R., HAY, M., and BISHOP, V. S. 1990. Neurons in area postrema mediate vasopressin-induced enhancement of the baroreflex. Am. J. Physiol. **258**: H1943-H1946.

CROFTON, J. T., SHARE, L., SHADE, R. E., LEE-KWON, W. J., MANNING, M., and SAWYER, W. H. 1979. The importance of vasopressin in the development and maintenance of DOCA-salt hypertension in the rat. Hypertension **1**: 31-38.

DATAR, S., CHIU, E. K. Y., and MCNEILL, J. R. 1985. Reflexes fail to reduce pressor activity of vasopressin in spontaneous hypertension. Am. J. Physiol. **248**: H49-H54.

DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., and WARNER, T. D. 1988. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. Proc. Natl. Acad. Sci. USA **85**: 9797-9800.

DENG, L. Y. and SCHIFFRIN, E. L. 1992. Effects of endothelin on resistance arteries of DOCA-salt hypertensive rats. Am. J. Physiol. **262**: H1782-H1787.

DIBONA, G. F. 1982. The functions of the renal nerve. Rev. Physiol. Biochem. Pharmacol. **94**: 75-181.

DOHI, Y., HAHN, A. W. A., BOULANGER, C. M., BUHLER, F. R., and LUSCHER, T. F. 1992. Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensive rat resistance arteries. Hypertension **19**: 131-137.

DOUCET, J., GONZALEZ, W., and MICHEL, J. B. 1996. Endothelin antagonists in salt-dependent hypertension associated with renal insufficiency. J. Cardiovasc. Pharmacol. **27**: 643-651.

DU VIGNEAUD, V., GISH, D. I., and KATSOYANNIS, P. G. 1954. A synthetic preparation prossessing biological properties associated with arginine-vasopressin. J. Am. Chem. Soc. **76**: 4751-4752.

EBERT, T. J., COWLEY, A. W.,JR., and SKELTON, M. 1986. Vasopressin reduces cardiac function and augments cardiopulmonary baroreflex resistance increases in man. J. Clin. Invest. **77**: 1136-1142.

EMERSON, T. 1966. Effects of angiotensin, epinephrine, noreprinephrine, and vasopressin on venous return. Am. J. Physiol. **210**: 933-942.

EMMELUTH, C. and BIE, P. 1992. Effects, release and disposal of endothelin 1 in conscious dogs. Acta Physiol. Scand. **146**: 197-204.

EMORI, T., HIRATA, Y., OHTA, K., KANNO, K., EGUCHI, S., IMAI, T., SHICHIRI, M., and MARUMO, F. 1991. Cellular mechanism of endothelin-1 release by angiotensin and vasopressin. Hypertension **18**: 165-170.

EMORI, T., HIRATA, Y., IMAI, T., EGUCHI, S., KANNO, K., and MARUMO, F. 1993. Cellular mechanism of natriuretic peptides-induced inhibition of endothelin-1 biosynthesis in rat endothelial cells. Endocrinology **133**: 2474-2480.

ESSIG, M., DUSSAULE, J-C., VANDERMEERSCH, S., CHATZIANTONIOU, C., and ARDAILLOU, R. 1997. Modulation by angiotensin II of endothelial cell control of DNA synthesis in human mesangial cells. Nephron **75**: 303-309.

FARINI, F. 1913. Diabete insipido ed opoterapia. Gazz. Osped. Clin. **34**: 1135-1139.

FILEP, J., FROLICH, J. C., and FEJES-TOTH, G. 1985. Evidence against a vasopressor role of ADH in malignant DOC-saly Hypertension. clin. and exper. **A7(10)**: 1457-1470.

FILEP, J., FROLICH, J. C., and FOLDES-FILEP, E. 1987. Role of AVP in malignant DOC-salt hypertension: studies using vascular and antidiuretic antagonists. Am. J. Physiol. **253**: F952-F958.

FOLKOW, B., HALLBACK, M., LUNDGREN, Y., SIVERSTSSON, R., and WEILL, L. 1973. Importance of adaptive changes in vascular design for establishment of primary hypertension studied in man and in spontaneously hypertensive rats. Circ. Res. **32/33 (suppl. I)**: I-2-I-16.

FOLKOW, B. 1982. Physiological aspects of primary hypertension . Physiol. Rev. **62**: 347-504.

FRANCHINI, K. G. and COWLEY, A. W.,JR. 1996. Sensitivity of the renal medullary circulation to plasma vasopressin. Am. J. Physiol. **271**: R647-R653.

FRIEDEN, J. and KELLER, A. D. 1954. The release of vasopressin in response to haemorrhage and its role in the mechanism of blood pressure regulation. Circ. Res. **2**: 214-220.

FRIEDMAN, S. H., FRIEDMAN, C. L., and NAKASHIMA, M. 1960. Accelerated appearance of DCA hypertension in rats treated with pitressin. Endocrinology **67**: 752-759.

FUJII, A. M. and VATNER, S. F. 1987. Baroreflex mechanisms buffering ? adrenergic antagonists in conscious dogs. Am. J. Physiol. **253**: H728-H736.

FUJITA, K., MATSUMURA, Y., KITA, S., MIYAZAKI, Y., HISAKI, K., TAKAOKA, M., and MORIMOTO, S. 1995. Role of endothelin-1 and the ET_A receptor in the maintenance of deoxycorticosterone acetate-salt-induced hypertension. Br. J. Pharmacol. **114**: 925-930.

FUKURODA, T., FUJIKAWA, T., OZAKI, S., ISHIKAWA, K., YANO, M., and NISHIKIBE, M. 1994. Clearance of circulating endothelin-1 by ETB receptors in rats. Biochem. Biophys. Res. Commun. **199**: 1461-1465.

GALATIUS-JENSEN, S., WROBLEWSKI, H., WMMELUTH, C., BIE, P., HAUNSO, S., and KASTRUP, J. 1996. Plasma endothelin in congestive heart failure: effect of the ACE inhibitor, fosinopril. Cardiovasc. Res. **32**: 1148-1154.

GANTEN, U., RASCHER, W., LANG, R. E., DIETZ, R., RETTIG, R., UNGER, T., TAUGNER, R., and GANTEN, D. 1983. Development of a new strain of spontaneously hypertensive rats homozygous for hypothalamic diabetes insipidus. Hypertension **5 (2 Pt 2)**: I119-I128.

GARCIA-VILLALON, A. L., GARCIA, J. L., FERNANDEZ, N., MONGE, L., GOMEZ, B., and DIEGUEZ, G. 1996. Regional differences in the arterial response to vasopressin: role of endothelial nitric oxide. Br. J. Pharmacol. **118**: 1848-1854.

GARDINER, S. M., COMPTON, A. M., KEMP, P. A., and BENNETT, T. 1991. The effects of phosphoramidon on the regional haemodynamic responses to human proendothelin (1-38) in conscious rats. Br. J. Pharmacol. **103**: 2009-2015.

GELLAI, M., FLETCHER, T., PULLEN, M., and NAMBI, P. 1996. Evidence for the existence of endothelin-B receptor subtypes and their physiological roles in the rat. Am. J. Physiol. **271**: R254-R261.

GILLESPIE, M. N., OWASOYO, J. O., MCMURTRY, I. F., and O'BRIEN, R. F. 1986. Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture. J. Pharmacol. Exp. Ther. **236**: 339-343.

GOMEZ-SANCHEZ, E. P., ZHOU, M., and GOMEZ-SANCHEZ, C. E. 1996. Mineralocorticoids, salt and high blood pressure. Steroids **61**: 184-188.

GOTO, K., KASUYA, Y., MATSUKI, N., TAKUWA, Y., KURIHARA, H., ISHIKAWA, T., KIMURA, S., YANAGISAWA, M., and MASAKI, T. 1989. Endothelin activates the dihydropyridine sensitive, voltage dependent Ca^{2+} channel in vascular smooth muscle. Proc. Natl. Acad. Sci. USA **86**: 3915-3918.

GRAF, B. M., FISCHER, B., MARTIN, E., BOSNJAK, Z. J., and STOWE, D. F. 1997. Differential Effect of Arginine Vasopressin on Isolated Guinea Pig Heart Function During Perfusion at Constant Flow and Constant Pressure. J. Cardiovasc. Pharmacol. **29**: 1-7.

GRATTON, J. P., COURNOYER, G., LOFFLER, B. M., SIROIS, P., and D'ORLEANS-JUSTE, P. 1997. ET_B receptor and nitric oxide synthase blockade induce BQ-123-sensitive pressor effects in the rabbits. Hypertension **30**: 1204-1209.

GREENWAY, C. V. and LAUTT, W. W. 1986. Blood volume the venous system preload and cardiac output. Can. J. Physiol. Pharmacol. **64**: 383-387.

GROSS, P. A, TRAVIS, V. L, HORWITZ, L., SCHRIER, R. W, and ANDERSON, R. J 1982. Effect of Desmopressin induced water retention on systemic hemodynamics in rat. Am. J. Physiol. **243**: H934-H940.

GUO, G. B. and ABBOUD, F. M. 1984. Angiotensin II attenuates baroreflex control of heart rate and sympathetic activity. Am. J. Physiol. **246**: H80-H89.

GUTKIND, S. J., KURIHARA, M., and SAAVEDRA, J. M. 1988. Increased angiotensin II receptors in brain nuclei of DOCA-salt hypertensive rats. Am. J. Physiol. **255**: H646-H650.

GUYTON, A. C. and HALL, J. E. 1996. The pituitary hormones and their control by the hypothalamus. In: Textbook of Medical Physiology, 9th Ed., 933-944. Philadelphia, W. B. Saunders company.

HALL, J. E., MONTANI, J. P., WOODS, L. L., and MIZELLE, H. L. 1986. Renal escape from vasopressin: role of pressure diuresis. Am. J. Physiol. **250**: F907-F916.

HAN, S. P., TRAPANI, A. J., FOK, K. F., WESTFALL, T. C., and KNUEPFER, M. M. 1989. Effects of endothelin on regional haemodynamics in conscious rats. Eur. J. Pharmacol. **159**: 303-305.

HASSER, E. M. and BISHOP, V. S. 1990. Reflex effect of vasopressin after blockade of V1 receptors in the area postrema. Circ. Res. **67,No2**: 265-271.

HAYNES, W. G., DAVENPORT, A. P., and WEBB, D. J. 1993. Endothelin: progress in pharmacology and physiology. Trends in Pharmacological Sciences **14**: 225-228.

HAYNES, W. G. and WEBB, D. J. 1993. Endothelium-dependent modulation of responses to endothelin-1 on human veins. Clin. Sci. **84**: 427-433.

HAYNES, W. G. and WEBB, D. J. 1994. Contribution of endogenous generation of endothelin-1 to basal vascular tone. Lancet **270**: 15262-15268.

HAYNES, W. G., FERRO, C. J., O'KANE, K. P. J., SOMERVILLE, D., LOMAX, C. C., and WEBB, D. J. 1996. Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. Circulation **93**: 1860-1870.

HEBERT, S. C., CULPEPPER, R. M., and ANDREOLI, T. E. 1981. NaCl transport in mouse medullary thick ascending limbs. I. Funtional nephron heterogeneity and ADH-stimulated NaCl cotransport. Am. J. Physiol. **241 (4)**: F412-F431.

HENDRICKX, G. R., BOETTCHER, D. H., and VATNER, S. F. 1976. Effects of angiotensin, vasopressin and methoxamine on cardiac function and blood flow distribution in conscious dogs. Am. J. Physiol. **231 (5)**: 1579-1587.

HERNANDEZ, I., INGLÉS, A. C., RUIZ, F., QUESADA, T., and CARBONELL, L. F. 1994. Cardiovascular effects of angiotensin II and vasopressin in intact awake rats. Gen. Pharmacol. **25**: 527-532.

HICKEY, K. A., RUBANYI, G. M., PAUL, R. J., and HIGHSMITH, R. F. 1985. Characterization of a coronary vasoconstrictor prioduced by cultured endothelial cells . Am. J. Physiol. **248**: C550-C556.

HIEDA, H. S. and GOMEZ-SANCHEZ, C. E. 1990. Hypoxia increases endothelin release in bovine endothelial cells in culture, but epinephrine, norepinephrine, serotonin, histamine and angiotensin II do not. Life Sci. **47**: 247-251.

HIRSCH, A. T., DZAU, V. J., MAJZOUB, J. A., and CREAGER, M. A. 1989. Vasopressin-mediated forearm vasodilation in normal humans. Evidence for a vascular vasopressin V2 receptor. Journal of Clinical Investigation **84**: 418-426.

HIWATARI, M., ABRAHAMS, J. M., SAITO, T., and JOHNSTON, C. I. 1986. Contribution of vasopressin to the maintenance of blood pressure in deoxycorticosterone-salt induced malignant hypertention in spontaneously hypertensive rats. Clinical Science **70**: 191-198.

HOFBAUER, K. G., MAH, S. C., BAUM, H. P., HANNI, H., WOOD, J. M., and KRAETZ, J. 1984. Endocrine control of salt and water exretion: the role of vasopressin in DOCA-salt hypertension. J. Cardiovasc. Pharmacol. **6**: S184-S191.

HOWELL, W. H. 1898. The physiological effects of extracts of the hypophysis cerebri and infundibular body. J. Exp. Med. **3**: 245-258.

IMAI, T., HIRATA, Y., EMORI, T., YANAGISAWA, M., MASAKI, T., and MARUMO, F. 1992. Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. Hypertension **19**: 753-757.

IMAI, T., HIRATA, Y., EMORI, T., and MARUMO, F. 1993. Heparin has an inhibitory effect on endothelin-1 synthesis and release by endothelial cells. Hypertension **21**: 353-358.

IMAI, Y., NOLAN, P. L., and JOHNSTON, C. I. 1983. Restoration of suppressed baroreflex sensitivity in rats with hereditary diabetes insipidus (Brattleboro Rats) by arginine-vasopressin and DDAVP. Circ. Res. **53**: 140-149.

IMAI, Y., ABE, K., SASAKI, S., MINAMI, N., MUNAKATA, M., SAKUMA, H., HASHIMOTO, J., NOBUNAGA, T., SEKINO, H., and YOSHINAGA, K. 1990. Cardiovascular depression and stabilization by central vasopressin in rats. Hypertension **15**: 291-300.

IMAI, Y., KIM, C. Y., HASHIMOTO, J., MINAMI, N., MUNAKATA, M,, and ABE, K. 1996. Role of vasopressin in neurocardiogenic responses to hemorrhage in conscious rats. Hypertension **27**: 136-143.

IMAIZUMI, T., HARADA, S., HIROOKA, Y., MASAKI, H., MOMOHARA, M., and TAKESHITA, A. 1992. Effects of OPC-21268, an orally effective vasopressin V_1 receptor antagonist in humans. Hypertension **20**: 54-58.

INOUE, A., YANAGISAWA, M., KIMURA, S., KASUYA, Y., MIYAUCHI, T., GOTO, K., and MASAKI, T. 1989. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three seperate genes. Proc. Natl. Acad. Sci. USA **86**: 2863-2867.

INTENGAN, H. D., HE, G., and SCHIFFRIN, E. L. 1998. Effect of vasopressin antagonism on structure and mechanics of small arteries and vascular expression of endothelin-1 in deoxycorticosterone acetate salt hypertensive rats. Hypertension **32**: 770-777.

ITAYA, Y., SUZUKI, H., MATSUKAWA, S., KONDO, K., and SARUTA, T. 1986. Central renin-angiotensin system and the pathogenesis of DOCA-salt hypertension in rats. Am. J. Physiol. **251**: H261-H268.

IWAMURO, Y., MIWA, S., MINOWA, T., ENOKI, T., ZHANG, X. F., ISHIKAWA, M., HASHIMOTO, N., and MASAKI, T. 1998. Activation of two types of Ca^{2+} permeable nonselective cation channels by endothelin-1 in A7r5 cells. Br. J. Pharmac. **124**: 1541-1549.

Jamison, R.L. and Kriz, W. (1982) *Urinary Concentrating Mechanism: Structure and Function*, New York, Oxford University Press.

JARD, S., GAILLARD, R. C., GUILLON, G., MARIE, J., SCHOENENBERG, P., MULLER, A. F., MANNING, M., and SAWYER, W. H. 1986. Vasopressin antagonists allow demonstration of a novel type of vasopressin receptor in the rat adenohypophysis. Mol. Pharmacol. **30**: 171-177.

JARD, S. 1998. Vasopressin receptors - A historical survey. Adv. Exp. Med. Biol. **449**: 1-13.

JOUGASAKI, M., SCHIRGER, J. A., SIMARI, R. D., and BURNETT, J. C.,JR. 1998. Autocrine Role for the Endothelin-B Receptor in the Secretion of Adrenomedullin. Hypertension **32**: 917-922.

KATUSIC, Z. S., SHEPHERD, J. T., and VANHOUTTE, P. M. 1984. Vasopressin causes endothelium-dependent relaxations of the canine basilar artery. Circ. Res. **55**: 575-579.

KATUSIC, Z. S. 1992. Endothelial L-Arginine pathway and regional cerebral arterial reactivity to vasopressin . Am. J. Physiol. **262**: H1557-H1562.

KIDO, T., SAWAMURA, T., HOSHIKAWA, H., D'ORLEANS-JUSTE, P., DENAULT, J. B., LEDUC, R., KIMURA, J., and MASAKI, T. 1997. Processing of proendothelin-1 at the C-terminus of big endothelin-1 is essential for proteolysis by endothlin-converting enzyme-1 in vivo. Eur. J. Biochem. **244**: 520-526.

KIMURA, S., KASUYA, Y., SAWAMURA, T., SHINIMI, O., SUGITA, Y., YANAGISAWA, M., GOTO, K., and MASAKI, T. 1989. Conversion of big endothelin 1 to 21 residue endothelin 1 is essential for expression of full vasoconstrictor activity: structure activity relationships of big endothelin 1. J. Cardiovasc. Pharmacol. **13 (Suppl. 5)**: S5-7-S18.

KING, A. J., BRENNER, B. M., and ANDERSON, S. 1989. Endothelin: a potent renal and systemic vasoconstrictor peptide. Am. J. Physiol. **256**: F1051-F1058.

KIRCHNER, K. A., SCANLON, P. H.,JR., and DZIELAK, D. J. 1993. Endothelium derived relaxing factor responses in DOCA salt hypertensive rats. Am. J. Physiol. **265**: R568-R572.

KLOOG, Y. and SOKOLOVSKY, M. 1989. Similarities in the mode and sites of action of sarafotoxins and endothelins. Trends Pharmacol. Sci. **10**: 212-214.

KOHAN, D. E. 1993. Endothelins in the kidney: physiology and pathophysiology. American Journal of Kidney Diseases **22**: 493-510.

KOHAN, D. E. and HUGHES, A. K. 1993. Autocrine role of endothelin-1 in rat IMCD: inhibition of AVP-induced cAMP accumulation. Am. J. Physiol. **265**: F126-F129.

KOHAN, D. E. and PADILLA, E. 1993. Osmolar regulation of endothelin-1 production by rat inner medullary collecting duct. J. Clin. Invest. **91**: 1235-1240.

KOHAN, D. E., PADILLA, E., and HUGHES, A. K. 1993. Endothelin B receptor mediates ET-1 effects on cAMP and PGE2 accumulation in rat IMCD. Am. J. Physiol. **256**: F670-F676.

KOHNO, M., KANO, H., HORIO, T., YOKOKAWA, K., YASUNARI, K., and TAKEDA, T. 1995. Inhibition of endothelin production by adrenomedullin in vascular smooth muscle cells. Hypertension **25**: 1185-1190.

Krogh, A. (1922) *The anatomy and physiology of capillaries*, 156-167, New Haven, Conn., Yale University Press.

KURIHARA, H., YOSHIZUMI, M., SUGIYAMA, T., TAKAKU, F., YANAGISAWA, M., MASAKI, T., HAMAOKI, M., KATO, H., and YAZAKI, Y. 1989. Transforming growth factor beta stimulates the expression of endothelin mRNA from vascular endothelial cells. Biochem. Biophys. Res. Commun. **159**: 1435-1440.

KURIHARA, Y., KURIHARA, H., SUZUKI, H., KODAMA, T., MAEMURA, K., and NAGAI, R. 1994. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature **368**: 703-710.

LAFFERTY, H. M., GARCIA, D. L., and RENNKE, H. G. 1991. Anemia ameliorates progressive renal injury in experimental DOCA salt hypertension. J. Am. Soc. Nephrol. **1**: 1180-1185.

LAMPRECHT, R., ST.RICHARDSON, J., WILLIAMS, R. B., and KOPIN, I. J. 1977. 6-Hydroxydopamine destruction of central adrenergic neurones prevents or reverses developing DOCA-salt hypertension in rats. J. Neurol. Trans. **40**: 149-158.

LARIVIERE, R., ST-LOUIS, J., SCHIFFRIN, E. L. 1988. Vascular binding sites and biological activity of vasopressin in DOCA-salt hypertensive rats. J. Hypertens. **6**: 211-217.

LARIVIERE, R., DAY, R., and SCHIFFRIN, E. L. 1993a. Increased expression of endothelin-1 gene in blood vessels of deoxycorticosterone acetate-salt hypertensive rats. Hypertension **21**: 916-920.

LARIVIERE, R., THIBAULT, G., and SCHIFFRIN, E. L. 1993b. Increased Endothelin-1 Content in Blood Vessels of Deoxycorticosterone Acetate-Salt Hypertensive but not in Spontaneously Hypertensive Rats. Hypertension **21**: 294-300.

LARIVIERE, R., DENG, L.Y., DAY, R., SVENTEK, P., THIBAULT, G., and SCHIFFRIN, E. L. 1995. Increased endothelin-1 gene expression in the endothelium of coronary arteries and endocardium in the DOCA-salt hypertensive rat. J. Mol. Cell. Cardiol. **27**: 2123-2131.

LAUTT, W. W. 1989. Resistance or Conductance for Expression of Arterial Vascular Tone. Microvasc. Res **37**: 230-236.

LAUTT, W. W. and MCQUAKER, J. E. 1989. Methodological approach to pharmacodynamic calculations of vascular responses in vivo. Proc. West. Pharmacol. Soc. **32**: 227-230.

LI, J. S., LARIVIèRE, R., and SCHIFFRIN, E. L. 1994. Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats: Evidence for a role of endothelin in vascular hypertrophy. Hypertension **24**: 183-188.

LI, J. S., SVENTEK, P., and SCHIFFRIN, E. L. 1996. Effect of antihypertensive treatment and N omega nitro L arginine methyl ester on cardiovascular structure in deoxycorticosterone acetate salt hypertensive rats. J. Hypertens. **14**: 1331-1339.

LIARD, J. F., DERIAZ, O., TSCHOPP, M., and SCHOUN, J. 1981. Cardiovascular dffects of vasopressin infused into the vertebral circulation of conscious dogs. Clin. Sci. **61**: 345-347.

LIARD, J. F. 1988. Vasopressin antagonists and their use in animal studies. Kidney Int. **34 (suppl 26)**: S-43-S47.

LIARD, J. F. 1994. L-NAME antagonizes vasopressin V2-induced vasodilatation in dogs. Am. J. Physiol. **266**: H99-H106.

LIN, H. Y., KAJI, E. H., WINKEL, G. K., IVES, H. E., and LODISH, H. F. 1991. Cloning and functional expression of a vascular smooth muscle endothelin receptor. Proc. Natl. Acad. Sci. USA **88**: 3185-3189.

LLUCH, S., CONDE, M. V., DIEQUEZ, G., LOPEZ DE PABLO, A. L., GONZALEZ, M. L., ESTRADA, C., and GOMEZ, B. 1984. Evidence for the direct effect of vasopressin on human and goat crebral arteries. J. Pharmacol. Exp. Ther. **228**: 749-755.

LOPEZ-FARRE, A., MONTANES, I., MILLAS, I., and LOPEZ-NOVA, J. M. 1989. Effect of endothelin on renal function in rats. Eur. J. Pharmacol. **163**: 187-189.

LOTE, C. J., THEWLES, A., and WOOD, J. A. 1989. Vasopressin -induced natriuresis in the conscious rat: role of blood pressure, renal prostaglandin synthesis and the peptide ANF. J. Physiol. **411**: 481-491.

LOWES, V. L., MCLEAN, L. E., KASTING, N. W., and FERGUSON, A. V. 1993. Cardiovascular consequences of microinjection of vasopressin and angiotensin II in the area postrema. Am. J. Physiol. **265**: R625-R631.

LÖFFLER, B. M., BREU, V., and CLOZEL, M. 1993. Effect of different endothelin receptor antagonists and of the novel non-peptide antagonist Ro 46-2005 on endothelin levels in rat plasma. FEBS Lett. **333**: 108-110.

MALEK, A. and IZUMO, S. 1992. Physiological fluid shear stress causes downregulation of endothelin-1 mRNA in bovine aortic endothelium. Am. J. Physiol. **263**: C389-C396.

MANNING, M. and SAWYER, W. H. 1986. Synthesis and receptor specifities of vasopressin antagonists. J. Cardiovasc. Pharmacol. **8 (suppl 7)**: 529-535.

MANNING, P. T., SCHWARTZ, D., KATSUBE, N. C., HOLMBERG, S. W., and NEEDLEMAN, P. 1985. Vasopressin-stimulated release of atriopeptin: Endocrine antagonists in fluid homeostasis. Science **229**: 395-397.

MARPLES, D., FROKIAER, J., and NIELSEN, S. 1999. Long-term regulation of aquaporins in the kidney. Am. J. Physiol. **276**: F331-F339.

MARTIN, D. S. and MCNEILL, J. R. 1987. Sensitivity of intestinal resistance vessels to vasopressin after ganglionic blockade in the conscious cat. J. Cardiovasc. Pharmacol. **9**: 368-374.

MARTIN, D. S. and MCNEILL, J. R. 1988. Pressor and intestinal vascular bed responses to vasopressin after cholinergic blockade. Am. J. Physiol. **254**: H45-H51.

MARTIN, D. S. and MCNEILL, J. R. 1990. Arginine vasopressin increases aparent whole body capacity in anesthetized cats. Am. J. Physiol. **258**: H722-H728.

MATSUGUCHI, H. and SCHMID, P. G. 1982. Pressor response to vasopressin and impaired baroreflex function in DOC-salt hypertension. Am. J. Physiol. **242**: H44-H49.

MATSUGUCHI, H., SHARABI, F. M., GORDON, F. J., JOHNSON, A. K., and SCHMID, P. G. 1982. Blood pressure and heart rate responses to microinjection of vasopressin into the nucleus tractus solitarius region of the rat. Neuropharmacology **21**: 687-693.

MATSUMURA, Y., FUJITA, K., MIYAZAKI, Y., TAKAOKA, M., and MORIMOTO, S. 1995. Involvement of endothelin-1 in deoxycorticosterone acetate salt-induced hypertension and cardiovascular hypertrophy. J. Cardiovasc. Pharmacol. **26 Suppl. 3**: S456-S458.

MATTSON, D. L., LU, S., ROMAN, R. J., and COWLEY, A. W.,JR. 1993. Relationship between renal perfusion pressure and blood flow in different regions of the kidney. Am. J. Physiol. **264**: R578-R583.

MAYINGER, B. and HENSEN, J. 1999. Nonpeptide vasopressin antagonists: A new group of hormone blockers entering the scene. Exp. Clin. Endocrinol. Diabetes **107**: 157-165.

MCCONNAUGHEY, M. M., MCCONNAUGHEY, J. S., and INGENITO, A. J. 1999. Practical considerations of the pharmacology of angiotensin receptor blockers. J. Clin. Pharmacol. **39**: 547-559.

MCNEILL, J. R., WILCOX, W. C., and PANG, C. C. Y. 1977. Vasopressin and angiotensin: reciprocal mechanisms controlling mesenteric conductance. Am. J. Physiol. **232(3)**: H260-H266.

MCNEILL, J. R. and PANG, C. C. Y. 1982. Effect of pentobarbital anesthesia and surgery on the control of arterial pressure and mesenteric resistance in cats: role of vasopressin and angiotensin. Can. J. Physiol. Pharmacol. **60**: 363-368.

MCNEILL, J. R. 1983. Redundant nature of the vasopressin and renin-angiotensin systems in the control of mesenteric resistance vessels of the conscious fasted cat. Can. J. Physiol. Pharmacol. **61**: 770-773.

MICHEL, H., BACKER, A., MEYER-LEHNERT, H., MIGAS, I., and KRAMER, H. J. 1993. Rat renal, aortic and pulmonary endothelin-1 receptors: effects of changes in sodium and water intake. Clin. Sci. **85**: 593-597.

MILLER, M. and MOSES, A. M. 1969. Radioimmunoassay of vasopressin with a comparison of immunological and biological activity in the rats posterior pituitary. Endocrinology **84**: 557-562.

MIMURA, Y., OGURA, T., YAMAUCHI, T., OTSUKA, F., OISHI, T., HARADA, K., HASHIMOTO, M., and OTA, Z. 1995. Effet of Vasopressin V1- and V2-Receptor Stimulation on Blood Pressure in DOCA-Salt hypertensive Rats. Acta Med Okayama **49(4)**: 187-194.

MIYAUCHI, T., SUZUKI, N., KURIHARA, T., YAMAGUCHI, I., SUGISHITA, Y., and MATSUMOTO, H. 1991. Endothelin-1 and endothelin-3 play different roles in acute and chronic alterations of blood pressure in patients with chronic hemodialysis. Biochem. Biophys. Res. Commun. **178**: 276-281.

MOHRING, B. and MOHRING, J. 1975. PLasma ADH in normal Long Evans rats and in Long Evans rats heterozygous and homozygous for hypothalamic diabetes insipidus. Life Sci. **17**: 1307-1314.

MOHRING, J., MOHRING, B., PETRI, M., and HAACK, D. 1977. Vasopressor role of ADH in the pathogenesis of malignant DOC hypertension. Am. J. Physiol. **232**: F260-F269.

MOHRING, J., GLANZER, K., MACIEL, J. A., DUSING, R., KRAMER, H. J., ARBOGAST, R., and KOCH-WESER, J. 1980. Greatly enhanced pressor response to antidiuretic hormone in patients with impaired cardiovacualr reflexes due to idiopathic orthostatic hypotension. J. Cardiovasc. Pharmacol. **2**: 367-376.

MONTANI, J. P., LIARD, J. F., SCHOUN, J., and MÖHRING, J. 1980. Hemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. Circ. Res. **47**: 346-355.

NABIKA, T., NARA, Y., IKEDA, K., ENDO, J., and YAMORI, Y. 1991. Genetic heterogeneity of the spontaneouly hypertensive rat. Hypertension **18**: 12-16.

NAITOH, M., SUZUKI, H., MURAKAMI, M., MATSUMOTO, A., ICHIHARA, A., NAKAMOTO, H., YAMAMURA, Y., and SARUTA, T. 1993. Arginine vasopressin produces renal vasodilation via V_2 receptors in conscious dogs. Am. J. Physiol. **265**: R934-R942.

NAKAMURA, K., SASAKI, S., MORIGUCHI, J., MORIMOTO, S., MIKI, S., KAWA, T., ITOH, H., NAKATA, T., TAKEDA, K., and NAKA, M. 1999. Central effects of endothelin and its antagonists on sympathetic and cardiovascular regulation in SHR-SP. J. Cardiovasc. Pharmacol. **33**: 876-882.

NAKAMURA, S., NARUSE, M., NARUSE, K., SHIODA, S., NAKAI, Y., and UEMURA, H. 1993. Colocalization of immunoreactive endothelin-1 and neurohypophysial hormones in the axons of the neural lobe of the rat pituitary. Endocrinology **132(2)**: 530-533.

NGUYEN, P. V., PARENT, A., DENG, L. Y., FLÜCKIGER, J-P., THIBAULT, G., and SCHIFFRIN, E. L. 1992. Endothelin vascular receptors and responses in DOCA-salt hypertensive rats. Hypertension **19(suppl II)**: II-98-II-104.

NUSSEY, S. S., ANG, V. T. Y., JENKINS, J. S., CHOWDREY, H. S., and BISSET, G. W. 1984. Brattleboro rat adrenal contains vasopressin. Nature **193**: 1297-1298.

O'BRIEN, R. F. and MCMURTRY, I. F. 1984. Endothelial cell supernatants contract bovine pulmonary artery rings. Am. Rev. Respir. Dis. **129**: A337.(Abstract)

O'BRIEN, R. F., ROBBINS, R. J., and MCMURTRY, I. F. 1987. Endothelial cells in culture produce a vasoconstrictor substance. J. Cell. Physiol. **132**: 263-270.

OHLSTEIN, E. H. and DOUGLAS, S. A. 1993. Endothelin-1 modulates vascular smooth muscle structure and vasomotion: implications in cardiovascular pathology. Drug. Dev. Res. **29**: 108-128.

OHNAKA, K., TAKAYANAGI, R., NISHIKAWA, M., HAJI, M., and NAWATA, H. 1993. Purification and characterisation of a phosphoramidon sensitive endothelin-converting enzyme in porcine aortic endothelium. . J. Biol. Chem. **268:26**: 759-766.

OISHI, R., MONOGUCHI, H., TOMITA, K., and MURUMO, F. 1991. Endothelin-1 inhibits AVP sitmulated osmotic water permeability in rat inner medullary collecting duct. Am. J. Physiol. **261**: F951-F956.

OKADA, H., KANNO, Y. S., and SARUTA, T. 1995. Effect of nonpeptide vasopressin receptor antagonists on developing, and established DOCA-salt hypertension in rats. Clin. and Exper. Hypertension **17(3)**: 469-483.

OKAMURA, T., AYAJIKI, K., FUJIOKA, H., and TODA, N. 1999. Mechanisms underlying arginine vasopressin-induced relaxation in monkey isolated coronary arteries. J. Hypertens. **17**: 673-678.

OLIVER, H. and SHAFER, E. A. 1895. On the physiological action of extracts of the pituitary body and certain other glandular organs. J. Physiol. **18**: 277-279.

ONOUE, H., KAITO, N., TOMII, M., TOKUDOME, S., NAKAJIMA, M., and ABE, T. 1994. Human basilar and middle cerebral arteries exhibit endothelium-dependent responses to peptides. Am. J. Physiol. Heart Circ. Physiol. **267**: H880-H886.

OSBORN, J. W.,JR., SKELTON, M. M., and COWLEY, A. W.,JR. 1987. Hemodynamic effects of vasopressin compared with angiotensin II in conscious rats. Am. J. Physiol. **252**: H628-H637.

OTA, M., CROFTON, J. T., and SHARE, L. 1994. Hemorrhage-induced vasopressin release in the paraventricular nucleus measured by in vivo microdialysis. Brain Res. **658**: 49-54.

PANG, C. C. Y., WILCOX, W. C., and MCNEILL, J. R. 1979. Hypophysectomy and saralasin on mesenteric vasoconstrictor response to vasopressin. Am. J. Physiol. **236(2)**: H200-H205.

PANG, C. C. Y. and TABRIZCHI, R. 1986. The effects of noradrenaline, D-HT 920, methoxamine, angiotensin II and vasopressin on mean circulatory filling pressure in conscious rats. Br. J. Pharmac. **89**: 389-394.

PATEL, K. P. 1991. Central alpha-2 adrenergic mechanisms in the renal nerve mediated natriuresis and diuresis produced by acute volume expansion. J. Auton. Nerv. Syst. **36**: 47-54.

PEULER, J. D., EDWARDS, G. L., SCHMID, P. G., and JOHNSON, A. K. 1990. Area postrema and differential reflex effects of vasopressin and phenylephrine in rats. Am. J. Physiol. **258**: H1255-H1259.

PHILLIPS, P. A., ABRAHAMS, J. M., KELLY, J., PAXINOS, G., GRZONKA, Z., MENDELSOHN, F. A. O., and JOHNSTON, C. I. 1988a. Localization of vasopressin binding sites in rat brain by in vitro autoradiograph using a radioiodinated V1 receptor antagonist. Neuroscience **27**: 749-761.

PHILLIPS, P. A., KELLY, J. M., ABRAHAMS, J. M., GRZONKA, Z., PAXINOS, G., MENDELSOHN, F. A. O., and JOHNSTON, C. I. 1988b. Vasopressin receptors in brain and kidney: studies using a radio-iodinated V1 receptor antagonist. J. Hypertens. **6 (Suppl 4)**: S550-S553.

PINTO, Y. M., PAUL, M., and GANTEN, D. 1998. Lessons from rat models of hypertension: from Goldblatt to genetic engineering. Cardiovasc. Res. **39**: 77-88.

PLUMPTON, C., FERRO, C. J., HAYNES, W. G., WEBB, D. J., and DAVENPORT, A. P. 1996. The increase in human plasma immunoreactive endothelin but not big endothelin-1 or its C-terminal fragment induced by systemic administration of the endothlin antagonist TAK-044. Br. J. Pharmacol. **119**: 311-314.

POLLOCK, D. M., DIVISH, B. J., and OPGENORTH, T. J. 1993. Stimulation of Endogenous Endothelin Release in the Anesthetized Rat. Journal of Cardiovascular Pharmacology **22 (suppl.8)**: S295-S298.

POLLOCK, M. D. and OPGENORTH, J. T. 1993. Evidence for endothelin-induced renal Vasoconstriction independent of ETA receptor activation. Am. J. Physiol. **264**: R222-R226.

PONEC, J., FÖLDES, O., and LICHARDUS, B. 1991. Failure to demonstrate natriuretic activity in the posterior pituitary after immunoneutralization of the vasopressin content. Horm. Metab. Res. **23**: 473-475.

PUFFENBERGER, E. G., HOSODA, K., WASHINGTON, S. S., NAKAO, K., DEWIT, D., YANAGISAWA, M., and CHAKRAVART, A. 1994. A missense mutation of the endothelin-B-receptor gene in multigenic Hirschsprung's disease. Cell **79**: 1257-1266.

QU, L., HAY, M., and BISHOP, V. S. 1997. Administration of AVP to the area postrema alters response of NTS neurons to afferent imputs. Am. J. Physiol. **272**: R519-R525.

RIPHAGEN, C. L. and PITTMAN, Q. J. 1989. Mechanisms underlying the cardiovascular responses to intrathecal vasopressin administration in rats. Can. J. Physiol. Pharmacol. **67 (4)**: 269-275.

ROBINSON, J. L. 1986. Effect of vasopressin and phenylephrine on artrial pressure and heart rate in conscious dogs. Am. J. Physiol. **251**: H253-H260.

ROHMEISS, P., BECKER, H., DIETRICH, R., LUFT, F., and UNGER, T. 1986. Vasopressin: mechanism of central cardiovascular action in conscious rats. J. Cardiovasc. Pharmacol. **8 (4)**: 689-696.

ROMAN, R. J., COWLEY, A. W.,JR., GARCíA-ESTAÑ, J., and LOMBARD, J. H. 1988. Pressure-diuresis in volume-expanded rats: Cortical and medullary hemodynamics. Hypertension **12**: 168-176.

ROSSI, N. F., O'LEARY, D. S., and CHEN, H. 1997. Mechanisms of centrally administered ET-1-induced increases in systemic arterial pressure and AVP secretion. Am. J. Physiol. **272(1 Pt 1)**: E126-E132.

RUBANYI, G. M. and POLOKOFF, M. A. 1994. Endothelins: Molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol. Rev. **46**: 325.

SAKAMOTO, A., YANAGISAWA, M., SAKURAI, T., TAKUWA, Y., YANAGISAWA, H., and MASAKI, T. 1991. Cloning and functional expression of human cDNA for ET_B endothelin receptor. Biochem. Biophys. Res. Commun. **178**: 656-663.

SAKURAI, T., YANAGISAWA, M., TAKUWA, Y., MIYAZAKI, H., and KIMURA, S. 1990. Cloning of a cDNA encoding a non-isopeptide-selective sub-type of the endothelin receptor. Nature **348**: 732-735.

SAKURAI, T., YANAGISAWA, M., INOUE, A., RYAN, U. S., KIMURA, S., GOTO, K., and MASAKI, T. 1991. cDNA cloning, sequence analysis and tissue distribution of rat preproendothelin-1 mRNA. Biochem. Biophys. Res. Commun. **175**: 44-47.

SAKURAI, T., YANAGISAWA, M., and MASAKI, T. 1992. Molecular characterisation of endothelin receptors. Trends in Pharmacological Sciences **16198**: 103-22639.

SANDS, J. M., NONOGUCHI, H., and KNEPPER, M. A. 1987. Vasopressin effects on urea and H_2O transport in inner medullary collecting duct subsegments. Am. J. Physiol. **253 (5 Pt 2)**: F823-F832.

SATIO, T. and YAJIMA, Y. 1982. Development of DOCA-salt hypertension in the brattleboro rat. NYAS 309-318.

SAWYER, W. H. and MANNING, M. 1988. The development of potent and specific vasopressin antagonists. Kidney Int. **34 (suppl 26)**: S-34-S-37.

SCHIFFRIN, E. L., THOME, F. S., and GENEST, J. 1983. Vascular angiotensin II receptors in renal and DOCA-salt hypertensive rats. Hypertension **5(Suppl.V)**: V-16-V-21.

SCHINI, V. B., KATUSIC, Z. S., and VANHOUTTE, P. M. 1990. Neurohypophyseal peptides and tachykinins stimulate the production of cyclic GMP in cultured porcine aortic endothelial cells. J. Pharmacol. Exp. Ther. **255**: 994-1000.

SCHMID, P,G. and PATEL, K. P. 1987. Cardiovascular effects of neurohypophyseal peptide. The Peptides **8**: 335-362.

SCHMID, P. G., GUO, G. B., and ABBOUD, F. M. 1985a. Different effects of vasopressin and angiotensin II on baroreflexes. Federation Proceedings **44**: 2388-2392.

SCHMID, P. G., GUO, G. B., AXTELLE, T. S., and ABBOUD, F. M. 1985b. Vasopressin Facilitates Baroreceptor Inhibition of Sympathetic Vasomotor Activity by More Than One Mechanism. In: Vasopressin, 11-20. Edited by Schrier, R. W., New York, Raven Press.

SCHUSTER, V. L., KOKKO, J. P., and JACOBSON, H. R. 1984. Interactions of lysyl-bradykinin and antidiuretic hormone in the rabbit cortical collecting tubule. J. Clin. Invest. **73**: 1659-1667.

SEO, B. and LÜSCHER, T. F. 1995. ET_A and ET_B receptors mediate contraction to endothelin-1 in renal artery of aging SHR: Effects of FR139317 and bosentan. Hypertension **25**: 501-506.

SERRADEIL-LE GAL, C., HERBERT, J. M., DELISEE, C., SCHAEFFER, P., RAUFASTE, D., GARCIA, C., DOL, F., MARTY, E., MAFFRAND, J. P., and LE FUR, G. 1995. Effect of SR-49059, a vasopressin V_{1a} antagonist, on human vascular smooth muscle cells. Am. J. Physiol. Heart Circ. Physiol. **268**: H404-H410.

SHARABI, F. M., GUO, G. B., ABBOUD, F. M., THAMES, M. D., and SCHMID, P. G. 1985. Contrasting effects of vasopressin on baroreflex inhibition of lumbar sympathetic nerve activity. Am. J. Physiol. **249 (5 Pt 2)**: H922-H928.

SIMONSON, M. S. and DUNN, M. J. 1990. Cellular signaling by peptides of the endothelin gene family. FASEB J. **4**: 2989-3000.

SIRVIO, M. L., METSARINNE, K., SAIJONMAA, , and FYRQUIST, F. 1990. Tissue distribution and half-life of ^{125}I-endothelin in the rats: importance of pulmonary clearance. Biochem. Biophys. Res. Commun. **167**: 1191-1195.

SMITH, M. J.,JR., COWLEY, A. W.,JR., GUYTON, A. C., and MANNING, R. D.,JR. 1979. Acute and chronic effects of vasopressin on blood pressure, electrolytes, and fluid volumes. Am. J. Physiol. **237**: F232-F240.

SMITH, P. M., LOWES, V. L., and FERGUSON, A. V. 1994. Circulating vasopressin influences area postrema neurons. Neuroscience **59**: 185-194.

SMITH, P. M. and FERGUSON, A. V. 1997. Vasopressin acts in the subfornical organ to decrease blood pressure. Neuroendocrinology **66**: 130-135.

SOGABE, K., NIREI, H., SHOUBO, M., NOMOTO, A., AO, S., NOTSU, Y., and ONO, T. 1993. Pharmacological Profile of FR139317, a Novel, Potent Endothelin ETA Receptor Antagonist. The Journal of Pharmacology and Experimental Therapeutics **264**: 1040-1046.

SONNTAG, M., SCHALIKE, W., and BRATTSTROM, A. 1990. Cardiovascular effects of vasopressin micro-injections into the nucleus tractus solitarii in normotensive and hypertensive rats. J. Hypertens. **8 (5)**: 417-421.

STARLING, E. H. and VERNEY, E. B. 1924. The secretion of urine as studied on the isolated kidney. Proc. Roy. Soc. Lond. **97**: 321-363.

STEBBINS, C. L., BONIGUT, S., LIVIAKIS, L. R., and MUNCH, P. A. 1998. Vasopressin acts in the area postrema to attenuate the exercise pressor reflex in anesthetized cats. Am. J. Physiol. **274**: H2116-H2122.

STEIN, P. D., HUNT, J. T., FLOYD, D. M., and MORELAND, S. 1994. The discovery of Sulfonamide Endothelin Antagonists and the Development of the Orally Active ETA Antagonist 5-(Dimethylamino)-1-naphthalensulfonamide. Journal of Medicinal Chemistry **37**: 229-331.

SUMPIO, B. E. and WIDMANN, M. D. 1990. Enhanced production of endothelium-derived contraction factor by endothelial cells subjected to pulsatile stretch. Surgery **108**: 277-281.

SUZUKI, N., MATSUMOTO, H., KITADA, C., MASAKI, T., and FUJINO, M. 1989. A sensitive sandwich-enzyme immunoassay for human endothelin . J. Immun. Methods. **118**: 245-250.

SUZUKI, N., MIYAUCHI, T., TOMOBE, Y., MATSUMOTO, H., GOTO, K., MASAKI, T., and FUJINO, M. 1990. Plasma concentrations of endothlin-1 in spontaneously hypertensive rats and DOCA-salt hypertensive rats. Biochem. Biophys. Res. Commun. **167**: 941-947.

SUZUKI, S., KAJIKURI, J., SUZUKI, A., and ITOH, T. 1991. Effects of endothelin-1 on endothelial cells in the porcine coronary artery. Circ. Res. **69**: 1361-1368.

SZCZEPANSKA-SADOWSKA, E., GRAY, D., and SIMON-OPPERMANN, C. 1983. Vasopressin in blood and third ventricle CSF during dehydration, thirst, and hemorrhage. Am. J. Physiol. **245**: R549-R555.

SZCZEPANSKA-SADOWSKA, E., STEPNIAKOWSKI, K., SKELTON, M. M., and COWLEY, A. W.,JR. 1994. Prolonged stimulation of intrarenal V_1 vasopressin receptors results in sustained hypertension. Am. J. Physiol. Regul. Integr. Comp. Physiol. **267**: R1217-R1225.

TAGAWA, T., IMAIZUMI, T., ENDO, T., SHIRAMOTO, M., HIROOKA, Y., ANDO, S., and TAKESHITA, A. 1993. Vasodilatory effect of arginine vasopressin is mediated by nitric oxide in human forearm vessels. J. Clin. Invest. **92**: 1483-1490.

TAHARA, A., TOMURA, Y., WADA, K., KUSAYAMA, T., TSUKADA, J., ISHII, N., YATSU, T., UCHIDA, W., and TANAKA, A. 1997. Effect of YM087, a Potent Nonpeptide Vasopressin Antagonist, on Vasopressin-Induced Hyperplasia and Hypertrophy of Cultured Vascular Smooth-Muscle Cell. J. Cardiovasc. Pharmacol. **30**: 759-766.

TAKAHASHI, K., GHATEI, M. A., JONES, P. M., MURPHY, J. K., LAM, H. C., O'HALLORAN, D. J., and BLOOM, S. R. 1991. Endothelin in human brain and pituitary gland: presence of immunoreactive endothelin, endothelin messenger ribonucleic acid, and endothelin receptors. J. Clin. Endocrinol. Metab. **72(3):** 693-699.

TALOM, R. T. and MCNEILL, J. R. 1997. Withdrawal-induced antihypertensive effect of vasopressin: role of the L-arginine/nitric oxide pathway. Can. J. Physiol. Pharmacol. **75**: 812-817.

TERADA, Y., TOMITA, K., NONOGUCHI, H., and MARUMO, F. 1992. Different localization of two types of endothelin receptor mRNA in microdessected rat nephron segments using reverse transcription and polymerase chain reaction assay. J. Clin. Invest. **90**: 107-112.

TIPAYAMONTRI, U., YOUNG, D. B., NUWAYHID, B. S., and SCOTT, R. E. 1987. Analysis of the cardiovascular effects of arginine vasopressin in conscious dogs. Hypertension **9**: 371-378.

TOBA, K., OUCHI, Y., LIANG, J., AKISHITA, M., and ORIMO, H. 1994. Effect of an intracerebroventricularly administered vasopressin V1 antagonist on blood pressure and heart rate in deoxycorticosterone-salt hypertensive rats. J. Auton. Nerv. Syst. **50**: 123-129.

TOMOBE, Y., YANAGISAWA, M., FUJIMORI, A., MASAKI, T., and GOTO, K. 1993a. Arginine-vasopressin increases the release of ET-1 into perfusate of rat mesenteric artery. Biochem. Biophys. Res. Commun. **191**: 654-661.

TOMOBE, Y., YANAGISAWA, M., FUJIMORI, A., MASAKI, T., and GOTO, K. 1993b. Arginine-vasopressin increases the release of ET-1 into perfusate of rat mesenteric artery. Biochem. Biophys. Res. Commun. **191**: 654-661.

TSCHUDI, M. R. and LÜSCHER, T. F. 1994. Characterization of contractile endothelin and angiotensin receptors in human resistance arteries: evidence for two endothelin and one angiotensin receptor. Biochem. Biophys. Res. Commun. **204**: 685-690.

UNDESSER, K. P., HASSER, E. M., HAYWOOD, J. R., JOHNSON, A. K., and BISHOP, V. S. 1985. Interactions of vasopressin with the area postrema in arterial baroreflex function in conscious rabbits. Circ. Res. **56**: 410-417.

UNGER, T., ROHMEISS, P., DEMMERT, G., GANTEN, D., LANG, R. E., and LUFT, F. C. 1986a. Differential modulation of the baroreceptor reflex by brain and plasma vasopressin. Hypertension **8 (6 Pt 2)**: II157-II162.

UNGER, T., ROHMEISS, P., DEMMERT, G., LUFT, F. C., GANTEN, D., and LANG, R. 1986b. Differential actions of neuronal and hormonal vasopressin on blood pressure and baroreceptor reflex sensitivity in rats. J. Cardiovasc. Pharmacol. **8**: S81-S86.

VALTIN, H. 1987. Physiological Effects of Vasopressin on the Kidney. In: Vasopressin: Principles and properties, 369-387. Edited by Gash, D. M. and Boer, G. J., New York, Plenum Press.

VANHOUTTE, P. M. and LÜSCHER, T. F. 1986. Periphereral mechanisms in cardiovascular regulation: Transmitters, receptors and the endothelium. In: Handbook of Hypertension, 96-123. Edited by Tarazi, R. C. and Zanchetti, A., Elsevier, Amsterdam.

VERHAAR, M. C., STRACHAN, F. E., NEWBY, D. E., CRUDEN, N. L., KOOMANS, H. A., and RABELINK, T. J. 1998. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. Circulation **97**: 752-756.

VIERHAPPER, H., WAGNER, O. F., NOWOTNY, P., and WALDHAUSL, W. 1990. Effect of endothelin-1 in man. Circulation **81**: 1415-1418.

VON DEN VELDEN, R. 1913. Die Nierenwirkung von Hypophysen extrakten beim Menschen. Berl. Klin. Wochenschr. **50**: 2083-2086.

WAGNER, H. N and BRAUNWALD, E. 1956. The pressor effect of the antidiuretic principle of the posterior pituitary in orthostatic hypotension. Journal of Clinical Investigation **35**: 1412-1418.

WAGNER, O. F., CHRIST, G., WOJTA, J., VIERHAPPER, H., PARZER, S., NOWOTNY, P. J., SCHNEIDER, B., WALDHAUSL, W., and BINDER, B. R. 1992. Polar secretion of endothelin-1 by cultured endothelial cells. J. Biol. Chem. **267**: 16066-16068.

WALKER, B. R., CHILDS, M. E., and ADAMS, E. M. 1988. Direct cardiac effects of vasopressin: role of V1- and V2-vasopressinergic receptors. Am. J. Physiol. **255**: H261-H265.

WALKER, B. R., HAYNES, J. JR., WANG, H. L., and VOELKEL, N. F. 1989. Vasopressin-induced pulmonary vasodilation in rats. Am. J. Physiol. **257**: H415-H422.

WANG, H., WILSON, N., and MCNEILL, J. R. 1993. Relationship of the antihypertensive effect of vasopressin withdrawal to sodium excretion in the Doca-salt hypertensive rat. Clin. Invest. Med. **16**: 348-357.

WANG, H. and MCNEILL, J. R. 1994. Renal function contributes to the antihypertensive effect of vasopressin in Doca-salt but not spontaneous hypertension. Am. J. Physiol. **267**: H1842-H1850.

WEBB, D. J., MONGE, J. C., RABELINK, T. J., and YANAGISAWA, M. 1998. Endothelin: new discoveries and rapid progress in the clinic. Trends Pharmacol. Sci. **19**: 5-8.

WEBB, M. L., DICKINSON, K. E. J., DELANEY, C. L., LUI, E. C. K., SERAFINO, R., COHEN, R. B., MONSHIZADEGAN, H., and MORELAND, S. 1992. The endothelin receptor antagonist, BQ-123, inhibits angiotensin II-induced contractions in rabbit aorta. Biochemical and Biophysical research Communitcations **185**: 887-892.

WEBB, M. L., BIRD, J. E., LIU, E. C. K., ROSE, P. M., SERAFINO, R., STEIN, P. D., and MORELAND, S. 1995. BMS-182874 is a Selective, Nonpeptide Endothelin ETA Receptor Antagonist. The Journal of Phamacology and Experimental Therapeutics **272**: 1124-1134.

WEBB, R. L., OSBORN, J. W.,JR., and COWLEY, A. W.,JR. 1986. Cardiovascular actions of vasopressin: baroreflex modulation in the conscious rat. Am. J. Physiol. **251**: H1244-H1251.

WEBER, K. T., SUN, Y., CAMPBELL, S. E., SLIGHT, S. H., GANJAM, V. K., GRIFFING, G. T., SWINFARD, R. W., and DIAZ-ARIAS, A. A. 1995. Chronic mineralocorticoid excess and cardiovascular remodeling. Steroids **60**: 125-132.

WELT, F. G. P. and RUTLEN, D. L. 1991. Effect of vasopressin on systemic capacity. Am. J. Physiol. **261**: H1494-H1498.

WEN, C., LI, M., and WHITWORTH, J. A. 1996. Validation of Transonic small animal flowmeter for measurement of cardiac output and regional blood flow in the rat. J. Cardiovasc. Pharmacol. **27**: 482-486.

WISLOCKI, G. B. and LEDUC, E. H. 1952. Vital staining of the hematoencephalic barrier by silver nitrate and trypan blue, and cytological comparisons of the neurohypophysis, pineal body, area postrema, intercolumnar tubercle and supraoptic creast. J. Comp. Neurol. **96**: 371-417.

WITTE, K., SCHNECKO, A., SCHMIDT, T., VOLL, C., KRANZLIN, B., and LEMMER, B. 1999. Cardiovascular risk, renal hypertensive damage, and effects of amlodipine treatment in transgenic TGR(mREN2)27 rats. Gen. Pharmacol. **33(5)**: 423-430.

XAVIER, F., YU, M., and MCNEILL, J. R. 1996. Validation of a flow-differential technigue for the recording of splenic blood-volume changes to vasoactive agents. Journal of Cardiovascular Pharmacology **28**: 605-610.

YAMADA, K., NAKAYAMA, M., NAKANO, H., MIMURA, N., and YOSHIDA, S. 1993. Endothelium-dependent vasorelaxation evoked by desmopressin and involvement of nitric oxide in rat aorta. Am. J. Physiol. **264 (2 Pt 1)**: E203-E207.

YAMAMOTO, T., KIMURA, T., OTA, K., SHOJI, M., INOUE, M., SATO, K., OHTA, M., and YOSHINAGA, K. 1992. Central effects of endothelin-1 on vasopressin release, blood pressure, and renal solute excretion. Am. J. Physiol. **262(6 Pt 1)**: E856-E862.

YAMAMOTO, T., YAMANE, Y., UMENDA, Y., YOSHIOKA, T., NAKAI, M., and IKEDA, M. 1984. Cardiovascular Hemodynamics and Vasopressin Blockade in DOCA-Salt Hypertensive Rats. Hypertension **6**: 394-407.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K., and MASAKI, T. 1988. A new potent vasoconstrictor peptide produced by vascular endothelial cells. Nature **332**: 411-415.

YOSHIMOTO, S., ISHIZAKI, Y., SASAKI, T., and MUROTA, S. I. 1991. Effect of carbon dioxide and oxygen on endothelin production by cultured procine cerebral and oxygen on endothelin production by cultured porcine cerebral endothlial cells. Stroke **22**: 378-383.

YU, M., GOPALAKRISHNAN, V., and MCNEILL, J. R. 1998. Hemodynamic Effect of a selective endothelin-a receptor antagonist in deoxycorticosterone acetate-salt hypertensive rats. Journal of Cardiovascular Pharmacology **31(Suppl.)**: S262-S264.

YUKIMURA, T., YAMASHITA, Y., MIURA, K., KIM, S., IWAO, H., TAKAI, M., and OKADA, T. 1994. Renal vasodilating and diuretic actions of a selective endothelin ET_B receptor agonist, IRL1620. Eur. J. Pharmacol. **264**: 399-405.

ZEIDEL, M. L., BRADY, H. R., KONE, G. C., GULLANS, S. R., and BRENNER, B. M. 1989. Endothelin, a peptide inhibitor of sodium Na^+-K^+-ATPase, in intact renal tubular endothelial cells. Am. J. Physiol. **257**: C1101-C1107.